

# Antidepressant Phenelzine Alters Differentiation of Cultured Human and Mouse Preadipocytes<sup>[S]</sup>

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## ABSTRACT

Change in body weight is a frequent side effect of antidepressants and is considered to be mediated by central effects on food intake and energy expenditure. The antidepressant phenelzine (Nardil) potently inhibits both monoamine oxidase and semicarbazide-sensitive amine oxidase activities, two enzymes that are highly expressed in adipose tissue, raising the possibility that it could directly alter adipocyte biology. Treatment with this compound is rather associated with weight gain. The aim of this work was to examine the effects of phenelzine on differentiation and metabolism of cultured human and mouse preadipocytes and to characterize the mechanisms involved in these effects. In all preadipocyte models, phenelzine induced a time- and dose-dependent reduction in differentiation and triglyceride accumulation. Modulation of lipolysis or glucose transport was not involved in phenelzine action. This effect was supported by the

reduced expression in the key adipogenic transcription factors peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ) and CCAAT/enhancer binding protein- $\alpha$ , which was observed only at the highest drug concentrations (30–100  $\mu$ M). The PPAR- $\gamma$  agonists thiazolidinediones did not reverse phenelzine effects. By contrast, the reduction in both cell triglycerides and sterol regulatory element-binding protein-1c (SREBP-1c) was detectable at lower phenelzine concentrations (1–10  $\mu$ M). Phenelzine effect on triglyceride content was prevented by providing free fatty acids to the cells and was partially reversed by overexpression of a dominant-positive form of SREBP-1c, showing the privileged targeting of the lipogenic pathway. When considered together, these findings demonstrate that an antidepressant directly and potently inhibits adipocyte lipid storage and differentiation, which could contribute to psychotropic drug side effects on energy homeostasis.

Weight change occurring during treatment of psychiatric disorders is a frequent side effect and may force patients to discontinue pharmacological treatment, thus putting them at the risk of relapse. Psychotropic drugs, including antidepressants, mood stabilizers, and antipsychotics, more often in-

crease but sometimes reduce body weight. Considering their widespread use, concern has arisen regarding treatment-associated weight gain and obesity-associated morbidities, such as type 2 diabetes and cardiovascular diseases (Després and Lemieux, 2006; Van Gaal et al., 2006).

However, the general mechanisms influencing body weight during treatment for mental disorders are still far to be elucidated and probably include disease-, improvement-, and drug-related factors. Many psychiatric disorders are associated per se with weight changes, most often with weight loss. Thus, it was believed that drug-induced weight gain merely reflects the restoration of normal body weight, but this issue

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**ABBREVIATIONS:** MAO, monoamine oxidase; ACC, acetyl-CoA carboxylase; aP2, adipocyte protein 2; C/EBP- $\alpha$ , CCAAT/enhancer binding protein- $\alpha$ ; [<sup>3</sup>H]DOG, 2-[1,2-<sup>3</sup>H]deoxy-D-glucose; ERK1/2, p42/p44 extracellular signal-regulated kinase; FAS, fatty acid synthase; G3PDH, glycerol-3-phosphate dehydrogenase; MEF, mouse embryonic fibroblasts; PPAR- $\gamma$ , peroxisome proliferator-activated receptor- $\gamma$ ; SREBP-1c, sterol regulatory element-binding protein-1c; SSAO, semicarbazide-sensitive amine oxidase; UCP1, uncoupling protein-1; DMEM, Dulbecco's modified Eagle's medium; PCR, polymerase chain reaction.

remains in debate (Zimmermann et al., 2003). Otherwise, it is well known that antidepressants can modulate weight by interfering with the function of specific central nervous system pathways involved in the regulation of appetite and food intake but also through variations in resting energy expenditure (Zimmermann et al., 2003). Thus, alterations in weight equilibrium induced by the major classes of antidepressants such as tricyclic agents or selective serotonin reuptake inhibitors, but also by monoamine oxidase (MAO) inhibitors used in a second-line strategy for severe depression, seem mainly related to the regulation of food intake and/or energy expenditure at the central nervous system level.

However, to our knowledge, the possibility that antidepressants could directly modify the biology of peripheral tissues involved in body energy homeostasis has not been documented. In particular, given the high expression levels of MAO and semicarbazide-sensitive amine oxidase (SSAO) in fat cells, it is quite conceivable that the antidepressants of the MAO inhibitor family could exert direct and specific effects on adipose tissue development or metabolism. This is reinforced by the observation that these amine oxidases could regulate major physiological functions in adipocyte.

It is now well established that in different species, SSAO expression and activity are largely induced during the adipose conversion process (Fontana et al., 2001; Mercier et al., 2001). SSAO activation by exogenous substrates activates glucose transport (Enrique-Tarancón et al., 1998), adipocyte differentiation (Fontana et al., 2001), and inhibits lipolysis (Morin et al., 2001; Visentin et al., 2003; Amant et al., 2005). All of these insulin-mimicking effects are related to SSAO-generated hydrogen peroxide production (Enrique-Tarancón et al., 2000). In vivo, SSAO substrates reduce hyperglycemia and improve glucose tolerance in animal models of diabetes (Marti et al., 2001). Although less documented, MAO activity is also present in adipose tissue (Tong et al., 1979) and is predominantly expressed in human fat cells as MAO-A, with a minor amount of MAO-B (Pizzinat et al., 1999). Tyramine, a preferential substrate for MAO, partially reproduces insulin effects on Akt phosphorylation, glucose transport, and adipose conversion (Fontana et al., 2001). In vivo studies also support the view that amine oxidase activities could interact with the control of energy balance, especially with lipid mobilization-deposition, because mitigation of obesity has been observed in rodents treated with amine oxidase inhibitors (Yu et al., 2004; Carpéné et al., 2007, 2008; Prévot et al., 2007).

These observations raise the possibility that amine oxidase inhibitors directly alter adipose tissue development and led us to examine the potential direct effects of several amine oxidase inhibitors on adipocyte differentiation or lipid accumulation in several preadipocyte culture models. We observed that in four independent models of adipocyte differentiation, the hydrazine derivative phenelzine (2-phenylethyl hydrazine, Nardil; Pfizer, New York, NY), an antidepressant with a potent MAO and SSAO inhibitory activity, dramatically prevented cell triglyceride accumulation and adipose conversion, a phenomenon related to a reduced expression of several key adipogenic transcription factors, such as PPAR- $\gamma$ , C/EBP- $\alpha$ , and SREBP-1c. These findings were in apparent discrepancy with body weight gain frequently observed during phenelzine treatment.

Our findings demonstrate that in addition to its central effect on food intake and energy expenditure, the antidepressant phenelzine can directly act on a preadipocyte to limit its development.

## Materials and Methods

**Subjects.** Adipose tissue was obtained from mesenteric fat depots from men or women (aged 16–77 years) undergoing abdominal surgery. Subjects were devoid of metabolic, endocrine, or cardiovascular diseases. The study was approved by local ethics committee, and an informed consent was given to the patients.

**Cell Culture.** Murine 3T3-F442A and 3T3-L1 preadipocytes and the murine brown preadipocyte T37i cell line (Zennaro et al., 1998) were grown until subconfluence conditions as described in the Supplemental Data. Preadipocytes were obtained from human adipose tissue fragments as described previously (Serazin-Leroy et al., 2000; Dieudonné et al., 2004) and then plated into cell culture dishes at a density of  $2$  to  $4 \times 10^4/\text{cm}^2$  in a DMEM/Ham's F-12 medium with 10% fetal calf serum and antibiotics. After an overnight culture, cells were washed four times. After 48 to 72 h, adipocyte differentiation was induced in a chemically defined medium including DMEM/Ham's F-12, antibiotics, 80 nM insulin, 0.2 nM triiodothyronine, and 10  $\mu\text{g}/\text{ml}$  transferrin, and for the first 2 days, 2.2  $\mu\text{M}$  troglitazone and 200  $\mu\text{M}$  3-isobutyl-1-methylxanthine.

The mouse embryonic fibroblasts (MEF), which constitutively express peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ) nuclear receptor (MEF-PPAR- $\gamma$ ) cells, were cultured as described previously (Davis et al., 2004). They were exposed or not to 10  $\mu\text{M}$  pioglitazone from confluence (day 0) or day 4 after confluence. Unless otherwise specified, murine cells were exposed to phenelzine from confluence. Human preadipocytes were treated with the drug when shifted in the serum-free medium.

**Morphological and Biochemical Determinations.** Morphological determination of cell lipid content, measurement of cell viability and proliferation, and determination of triglyceride content and glycerol-3-phosphate dehydrogenase (G3PDH) activity in preadipose or adipose cells are described in the Supplemental Data.

**Western Blot Analysis.** For Western blot analysis, cell extracts and protein electrophoresis were performed as mentioned in the Supplemental Appendix and Supplemental Table 1.

**Lipolysis Experiments.** Lipolysis was assessed as glycerol release from adherent 3T3-F442A adipocytes. Adipocytes were cultured without insulin for 24 h, then shifted for a 24-period in a serum-free medium including DMEM/Ham's F-12, antibiotics, and 0.1% fatty acid-free BSA. Thereafter, cells were cultured in the same medium in the absence or in the presence of 100  $\mu\text{M}$  phenelzine. Lipolysis experiments with or without 10  $\mu\text{M}$  (–)-isoproterenol or 10  $\mu\text{M}$  forskolin were performed as described in the Supplemental Data.

**Determination of 2-Deoxy-D-glucose Uptake.** Adipocytes were cultured and treated as for the lipolysis experiments. Uptake of glucose was determined using 2-[1,2- $^3\text{H}$ ]deoxy-D-glucose ([ $^3\text{H}$ ]DOG; ICN Biochemicals, Orsay, France), a nonmetabolizable analog of glucose. Glucose uptake is described in the Supplemental Data.

**Quantitative Real-Time PCR.** Total RNA was extracted by the method of Cathala et al. (1983), and quantitative real-time PCR was performed as described previously (Teixeira et al., 2006). Details on RNA analysis are given in the Supplemental Appendix and in Supplemental Table 2.

**Adenoviral Experiments.** The adenovirus vector encoding for the dominant-positive form of rat SREBP-1c, so-called Ad.SREBP-1c DP, was generated as mentioned previously (Foretz et al., 1999). SREBP-1c gene expression was under control of a cytomegalovirus promoter and a green fluorescent protein, coexpressed to monitor transfection efficiency. The adenovirus containing the major late promoter with no exogenous gene, called Ad.null, was used as a control. Adenoviral vectors were purified after propagation in the human em-

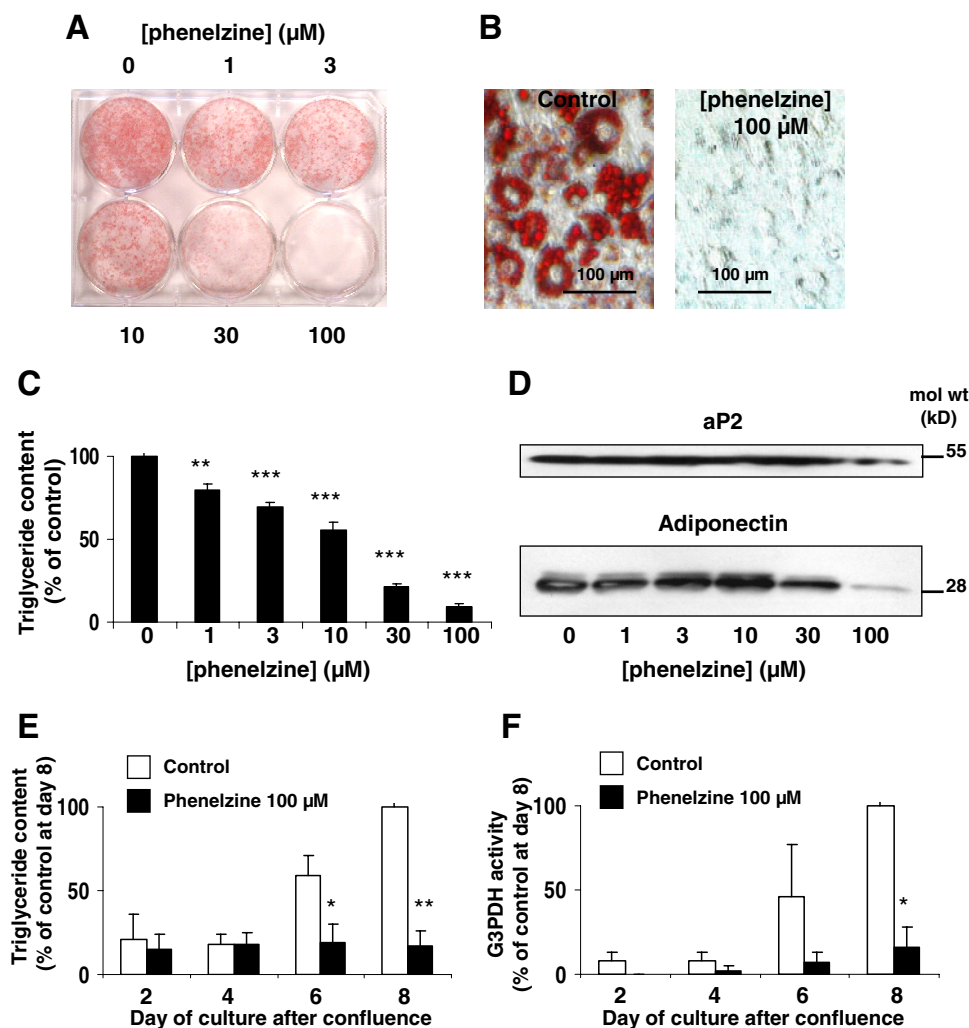
byronic kidney 293 cell line and stored at  $-80^{\circ}\text{C}$  until use. At confluence, adenofection was performed under serum-free conditions for 90 min at a multiplicity of infection of 500 (500 plaque-forming units/cell) that is known to achieve an optimal infection efficiency in 3T3 adipocytes (Le Lay et al., 2002). Cells were then refed in a serum-containing differentiating medium in the absence or in the presence of 50 or 100  $\mu\text{M}$  phenelzine. Similar efficiency in viral infection was assessed by green fluorescent protein expression. Microscopic analysis, cell triglyceride, G3PDH activity, and FAS and adiponectin expression were analyzed at day 7 after confluence.

**Statistical Analysis.** Results of quantitative analysis are presented as mean  $\pm$  S.E., as specified in the figure legends. In the dose-dependence and time-course experiments (triglyceride and G3PDH assays), a separate one-way analysis of variance was used with the selected concentrations or time as the independent variables and the percentage between drug-exposed and control samples as the dependent variable. Comparison of mean values between groups was analyzed with post hoc Tukey's honestly significant difference multiple comparison test. For lipolysis, glucose transport, and reverse-transcriptase PCR experiments, the statistical significance of differences between means was analyzed with a two-sided Student's *t* test. A *p* value  $<0.05$  was considered as the threshold of statistical significance.

## Results

### Phenelzine Is a Potent Inhibitor of Preadipocyte Differentiation and Triglyceride Accumulation. Confluent

3T3-F442A cells were cultured for various periods of time in the absence or presence of the drug, within a concentration range compatible with that detectable in the plasma of treated patients (available at <http://www.biopsychiatry.com/phenelzine/phenelzine-nardil>). After an 8-day treatment with various phenelzine concentrations, a slight decrease in lipid content was detectable from 1  $\mu\text{M}$  and was dramatic at 30  $\mu\text{M}$ , reaching a maximal effect at 100  $\mu\text{M}$  (Fig. 1A). This was confirmed by microscopic analysis showing that an 8-day exposure to 100  $\mu\text{M}$  phenelzine dramatically decreased the number and size of fat vacuoles compared with control cells (Fig. 1B). It is noteworthy that the reduction in lipid droplets occurs at concentrations where alterations in the spheric shape of adipocytes were not detectable. This observation suggested that at low concentrations, phenelzine preferentially altered the process of triglyceride accumulation, whereas a global reduction in the differentiation level occurred at higher concentrations. In agreement with this result, phenelzine induced a dose-dependent decrease in 3T3-F442A cell triglyceride content that was significant from 1  $\mu\text{M}$  and then continued to markedly decline with increasing doses (Fig. 1C), with a maximal 90% reduction at 100  $\mu\text{M}$  compared with control cells, giving a half-maximal effect at 20  $\mu\text{M}$ . Adiponectin and aP2 protein expressions were also examined by Western blot analysis in 3T3-F442A cells treated with various phenelzine concentrations (Fig. 1D). aP2



**Fig. 1.** Dose- and time-dependent inhibitory effect of phenelzine on 3T3-F442A differentiation. Confluent 3T3-F442A cells (day 0) were cultured for 8 days in the absence or in the presence of various concentrations of phenelzine. At day 8, cells were stained with oil Red O and photographed macroscopically (A) and under microscopy (B). C, at day 8, cell triglyceride content was tested. Results represent the mean  $\pm$  S.E. of eight separate determinations and are expressed as the percentage of the control value (mean, 0.34 g/liter). D, at day 8, cell extracts were prepared, and aP2 and adiponectin protein expression was tested by Western blot analysis. E, 3T3-F442A cells were cultured from confluence (day 0) in the absence or in the presence of phenelzine (100  $\mu\text{M}$ ). At days 2, 4, 6, and 8, cell triglyceride content was tested. F, cytosolic extracts were prepared in parallel, and G3PDH activity was measured. Results represent the mean  $\pm$  S.E. of four separate determinations and are expressed as the percentage of the control value at day 8 (0.52 g/liter and 566 nmol/min/mg for triglyceride content and G3PDH activity, respectively). \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ , phenelzine-treated versus control.



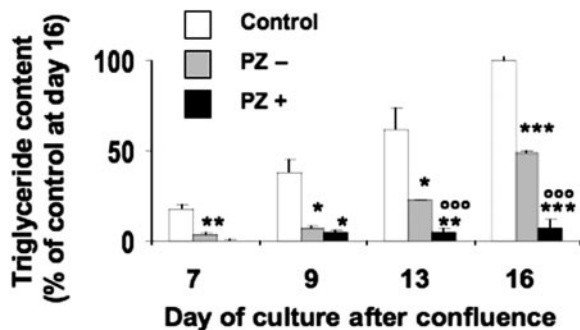
down-regulation was only observed at the highest 100  $\mu\text{M}$  phenelzine concentration. Adiponectin expression levels were maintained until a concentration of 10  $\mu\text{M}$  and were decreased from 30  $\mu\text{M}$ , with a maximal reduction at 100  $\mu\text{M}$ . The dose-dependent phenelzine-induced decrease in G3PDH activity that reflected the level of adipocyte differentiation had a pattern close to that of adiponectin (data not shown).

Confluent 3T3-F442A cells were maintained from confluence in the absence or in the presence of 100  $\mu\text{M}$  phenelzine, and cell extracts were prepared at intervals. At day 6 after confluence, phenelzine provoked a marked 3-fold decrease in cell triglyceride content (Fig. 1E). This effect was even more pronounced at day 8, with approximately a 6-fold reduction. A similar profile was observed for G3PDH activity, which was dramatically down-regulated by phenelzine in 3T3-F442A cells on day 8 after confluence (Fig. 1F).

The reversibility of phenelzine effect was also tested in FT3-F442A cells (Fig. 2). Long-term phenelzine exposure (100  $\mu\text{M}$ ) for 7 days from confluence markedly reduced cell triglyceride accumulation. When phenelzine treatment was pursued throughout the culture, cell triglyceride remained very low and maximally represented less than 10% of the control value at day 16 after confluence. On the contrary, phenelzine withdrawal from the culture medium at day 7 allowed 3T3-F442A cells to progressively recover a large part of their triglyceride content, although it remained significantly lower than that from control adipocytes.

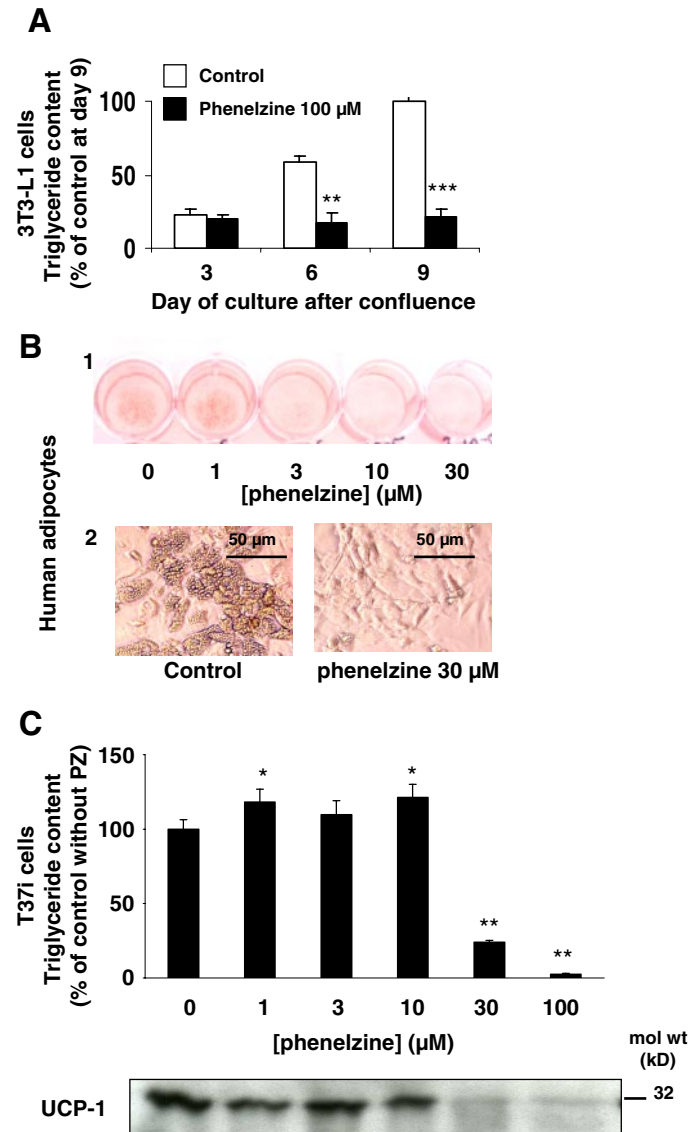
In addition, we examined the effect on fully mature adipocytes (day 8 after confluence) of an 8-day treatment with various concentrations of the drug (1–100  $\mu\text{M}$ ). Under these culture conditions, phenelzine displayed no significant effect on cell triglyceride content (Supplemental Table 3).

Similar effects of phenelzine were observed on three other models of adipogenesis (i.e., the murine 3T3-L1 white preadipose cell line, primary cultures of stromal vascular fraction derived from human subcutaneous abdominal adipose tissue, and the murine brown preadipose T37i cell line). For 3T3-L1 cells, triglyceride accumulation was markedly reduced by 100  $\mu\text{M}$  phenelzine at day 6 after confluence, reaching a maximal 80% reduction at day 9 (Fig. 3A). For primary cultures of human preadipocyte cultures in a chemically defined medium,



**Fig. 2.** Spontaneous reversibility of the phenelzine effect on 3T3-F442A adipocyte differentiation. Confluent 3T3-F442A cells (day 0) were cultured in the absence or presence of phenelzine (100  $\mu\text{M}$ ). At day 7, in cells previously exposed to phenelzine, the compound was either omitted (PZ-) or maintained (PZ+). At days 7, 9, 13, and 16, cell triglyceride content was assayed. Results represent the mean  $\pm$  S.E. of five to six separate determinations and are expressed as the percentage of the control value at day 16 (mean, 2.18 g/liter). \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ , phenelzine-treated versus control cells, whatever the sequence to drug exposure; °°,  $p < 0.001$ , PZ- cells compared with PZ+ cells.

phenelzine induced a dose-dependent reduction in Oil Red O staining (Fig. 3B) that was seen from 3  $\mu\text{M}$  and was maximal between 10 and 30  $\mu\text{M}$ . The effect of the drug on lipid accumulation was also illustrated by the dramatic decrease in the number and size and lipid droplets (Fig. 3B). Finally, we also examined the influence of phenelzine during the course of differentiation of the brown preadipocyte T37i cell line (Zennaro et al., 1998). Phenelzine induced a dramatic and dose-dependent decrease in triglyceride storage of T37i cells (Fig. 3C). In addition, the drug provoked a marked dose-



**Fig. 3.** Phenelzine inhibits differentiation of other preadipocyte models. A, confluent 3T3-L1 cells were cultured from day 0 in the absence or in the presence of phenelzine (100  $\mu\text{M}$ ). Cell triglyceride content was tested at days 3, 6, and 9. Results represent the mean  $\pm$  S.E. of four to five separate determinations and are expressed as the percentage of the control value at day 9 (mean, 0.37 g/liter). B, human preadipocytes were cultured in the absence or presence of various concentrations of phenelzine. At day 8 in a chemically defined medium, cells were photographed macroscopically after staining with oil Red O (B-1) and under microscopy without any coloration (B-2). C, the murine brown preadipose T37i line was cultured in the absence or in the presence of phenelzine (100  $\mu\text{M}$ ). At day 7, cells were stained with Oil Red O and photographed macroscopically and tested for triglyceride content (mean control value, 0.81 g/liter) and UCP1 expression. \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ , phenelzine-exposed versus control cells.

dependent down-regulation of UCP1 (Fig. 3C). Taken together, the observations drawn from four distinct models of adipogenesis indicate that phenelzine strongly inhibits the adipocyte differentiation process and has a preferential effect on cell triacylglycerol accumulation.

**Mechanisms Involved in Phenelzine Effects on Lipid Accumulation and Adipose Conversion.** We then attempted to elucidate the molecular mechanisms by which phenelzine altered adipose conversion and fat storage. We first ensured that phenelzine did not exert a direct cytotoxic effect. Microscopic analysis did not reveal any detectable cell lysis, whatever the cultured cell model. Moreover, cell viability was assessed in 3T3-F442A cells by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay and indicated that cell exposure to 100  $\mu$ M phenelzine for 8 days did not induce cytotoxicity ( $100 \pm 2.9$  and  $106.2 \pm 2.8\%$  in control and phenelzine-treated cells, respectively;  $n = 16$ ). This was confirmed by Trypan blue exclusion test (data not shown).

Phenelzine effects on adipocyte differentiation could implicate differences in cell proliferation rates. [ $^3$ H]Thymidine incorporation assays show that phenelzine exposure (100  $\mu$ M) for 48 h during clonal expansion slightly decreased cell proliferation by 30% (Supplemental Table 4). In addition, the same experiment performed in preconfluent cells did not reveal a significant phenelzine effect during the exponential growth phase.

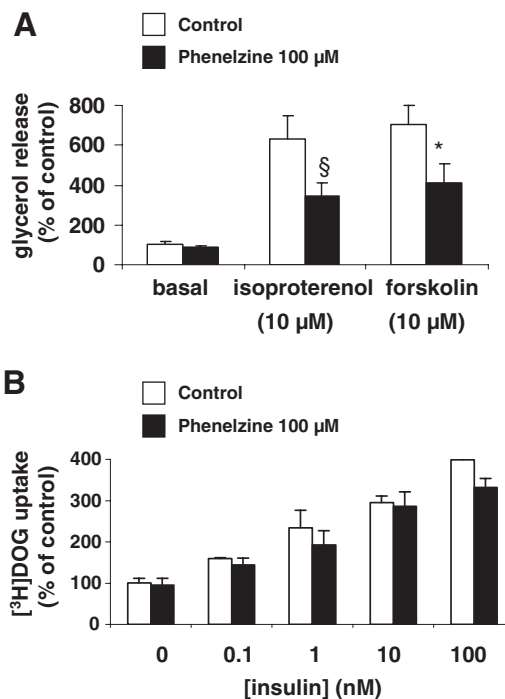
The inhibitory effect of phenelzine on cell fat accumulation could be related to an activation of triacylglycerol hydrolysis (i.e., lipolysis). Hence, mature 3T3-F442A adipocytes were treated for 48 h with 100  $\mu$ M phenelzine, and lipolysis was tested under basal conditions or in response to an optimal concentration of the  $\beta$ -adrenoceptor agonist (–)-isoproterenol (10  $\mu$ M) or of the adenylyl cyclase effector forskolin (10  $\mu$ M). As shown in Fig. 4A, phenelzine did not modify basal lipolysis. However, it induced an almost 2-fold reduction in (–)-isoproterenol- or forskolin-stimulated glycerol production.

Another hypothesis was that phenelzine could alter glucose availability, leading to a decreased fatty acid and triglyceride synthesis. Thus, we examined the influence of phenelzine on basal and insulin-stimulated glucose transport of 3T3-F442A adipocytes. Neither basal nor maximal insulin-activated [ $^3$ H]DOG transport was affected by a prior exposure to phenelzine (Fig. 4B). In addition, the antidepressant did not modify the EC<sub>50</sub> values of insulin for stimulating [ $^3$ H]DOG uptake.

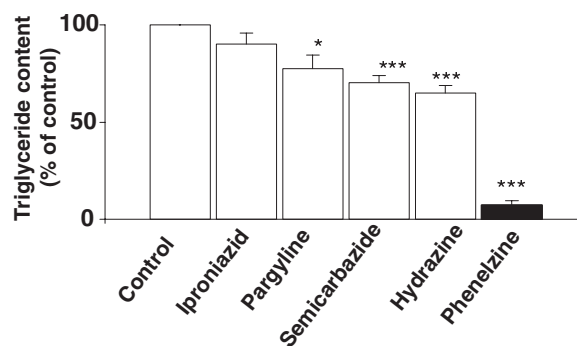
It was questionable whether phenelzine effect on cell triglyceride accumulation and adipocyte differentiation could be related to its inhibitory properties on amine oxidase activities. For this purpose, we compared the potencies of various amine oxidase inhibitors to modulate cell triglyceride content. Although pargyline and semicarbazide are MAO- and SSAO-selective inhibitors, respectively, iproniazid, hydrazine, and phenelzine inhibit both MAO and SSAO activities. Drugs were used at a concentration of 100  $\mu$ M because it has been shown that it maximally inhibits the related amine oxidase activity (Moldes et al., 1999; Mercier et al., 2003). Iproniazid did not display a significant effect on cell lipid accumulation (Fig. 5). Pargyline, semicarbazide, and hydrazine only provoked a weak but significant decrease in lipid fat stores. Thus, there was no clear relationship between the pharmacological properties of a given compound on

specific amine oxidase activities and its capacity to modulate adipocyte lipid storage.

We also investigated the influence of phenelzine on the intimate control of the adipocyte differentiation program. We tested the variations of the key adipogenic transcription fac-



**Fig. 4.** Effect of phenelzine on lipolysis and glucose uptake in mature 3T3-F442A cells. A, 8 days after confluence, 3T3-F442A cells were cultured in the absence or in the presence of phenelzine (100  $\mu$ M) for 48 h. Lipolysis was measured by the determination of glycerol release in the culture medium under basal conditions or in response to an optimal concentration of (–)-isoproterenol (10  $\mu$ M) or forskolin (10  $\mu$ M). Results represent the mean  $\pm$  S.E. of six separate determinations and are expressed as the percentage of the basal control value (mean, 65.2  $\mu$ M). B, 8 days after confluence, 3T3-F442A cells were cultured in the absence or in the presence of phenelzine (100  $\mu$ M) for 48 h. [ $^3$ H]DOG uptake was measured under basal conditions or in response to various concentrations of insulin. Results represent the mean  $\pm$  S.E. of four separate determinations and are expressed as the percentage of the control value (mean, 35 pmol/min/well).  $\S$ ,  $p = 0.052$ ;  $*$ ,  $p < 0.05$ , phenelzine-exposed versus control cells.



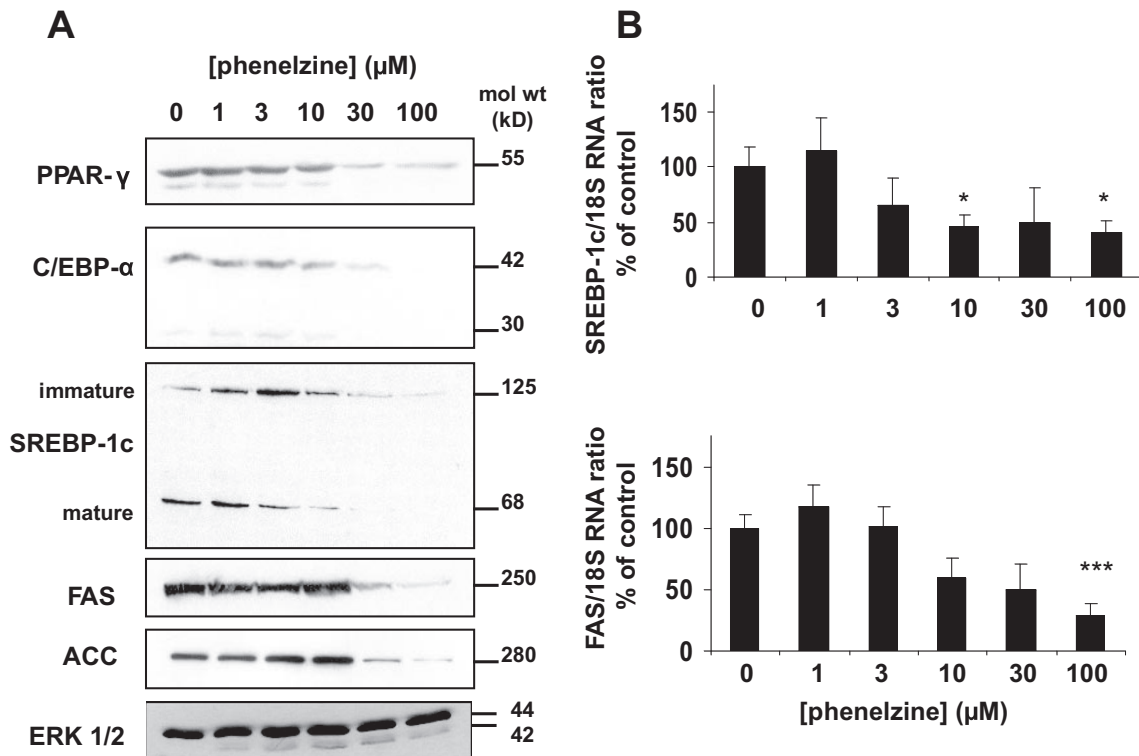
**Fig. 5.** Effect of long-term exposure various amine oxidase inhibitors on triglyceride accumulation of differentiating 3T3-F442A adipocytes. Confluent 3T3-F442A cells (day 0) were cultured for 8 days in the absence or in the presence of the indicated amine oxidase inhibitor (100  $\mu$ M). Cell extracts were prepared and assayed for triglyceride content. Results represent the mean  $\pm$  S.E. of 10 separate determinations and are expressed as the percentage of the control value (mean, 0.72 g/liter).  $*$ ,  $p < 0.05$ ;  $***$ ,  $p < 0.001$ , drug-exposed versus control cells.

tors PPAR- $\gamma$ , C/EBP- $\alpha$ , and SREBP-1c after an 8-day exposure from confluence to various drug concentrations (1–100  $\mu$ M) (Fig. 6A). PPAR- $\gamma$  expression was affected only at the two highest phenelzine concentrations (30 and 100  $\mu$ M), with a maximal 50% decrease. Likewise, C/EBP- $\alpha$  was down-regulated at 30 and 100  $\mu$ M. Of interest, the decrease in the immature large form of SREBP-1c was observed from 10  $\mu$ M phenelzine. The reduction in the transcriptionally active mature form of SREBP-1c was even detectable from 3  $\mu$ M, with virtually no expression from 30  $\mu$ M. As expected, the down-regulation in SREBP-1c expression induced a clear reduction of the lipogenic enzymes FAS and ACC, whose genes represent direct targets for the SREBP-1c transcription factors. As an internal control, no significant change in the p42/p44 ERK1/2 was detected in the presence of phenelzine. Moreover, phenelzine caused a clear dose-dependent decrease in SREBP-1c mRNA steady-state levels, with a profile close to that of protein expression (Fig. 6B). However, there was only approximately a maximal 3-fold reduction in SREBP-1c mRNA abundance, thus raising the possibility of translational or post-translational regulations of the transcription factor by the compound. As expected, the drug induced a reduction in FAS expression, whose gene represents a typical target gene of SREBP-1c (Fig. 6, A and B).

We also tested whether the inhibitory effect of phenelzine on cell triglyceride accumulation could be circumvented by directly providing exogenous fatty acids to the cells. Confluent 3T3-F442A cells were cultured with or without phenelzine, and in the absence or in the presence of Intralipid (100 mg/liter; Kabivitrum, Stockholm, Sweden) as an exogenous

source of fatty acids (Fig. 7). As expected, Intralipid alone provoked a moderate increase in cell triglyceride content. Overall, the phenelzine-induced decrease in cell triglycerides was prevented in the presence of Intralipid. Interestingly, Intralipid could not restore the inhibitory effect of the drug on G3PDH activity (Fig. 7B). These results suggest that although the phenelzine-induced blockade of the lipogenic pathway probably has a key role in the decrease in fat stores, other drug-mediated events also contribute to the alterations in the adipose conversion process.

We then examined whether the phenelzine-induced down-regulation in SREBP-1c expression was responsible, at least in part, for the reduction in cell triglyceride content. The ability of an adenovirus-driven expression of SREBP-1c to prevent the effects of phenelzine on cell lipid storage was investigated in 3T3-F442A cells. As observed previously in noninfected cells, phenelzine caused in 3T3-F442A cells infected with the Ad-null vector a dramatic and dose-dependent decrease in triglyceride accumulation (Fig. 8, A and B), G3PDH activity (Fig. 8C), and adiponectin and FAS expression (Fig. 8D). In cells infected with Ad-SREBP-1c DP cultured without phenelzine, and in agreement with previous works (Le Lay et al., 2002), there was a slight but not significant increase in lipid vacuoles and triglyceride accumulation (Fig. 8, A and B) that could be related to the induction of the lipogenic pathway reflected by the induction of FAS expression (Fig. 8D, lane 2 compared with lane 1). When cells were exposed to the maximal dose of 100  $\mu$ M phenelzine, infection with Ad.SREBP-1c only weakly reversed the drug effects on the morphological and biochemical markers of adipocyte dif-



**Fig. 6.** Dose-dependent inhibitory effect of phenelzine on protein expression and mRNA levels of adipogenesis markers in 3T3-F442A cells. Confluent 3T3-F442A cells (day 0) were cultured for 8 days in the absence or presence of various concentrations of phenelzine. A, at day 8, cell extracts were prepared, and PPAR $\gamma$ , C/EBP $\alpha$ , SREBP-1c (mature and immature forms), FAS, ACC, and ERK1/2 protein expression was tested by Western blot analysis using specific antibodies. B, at day 8, total RNA was prepared, and SREBP-1c and FAS mRNA levels were determined by quantitative real-time reverse-transcriptase PCR analysis and are normalized to the 18S RNA levels. Results represent the mean  $\pm$  S.E. of four to six separate determinations and are expressed as the percentage of the control value. \*,  $p < 0.05$ ; \*\*\*,  $p < 0.001$ , phenelzine-exposed versus control cells.



ferentiation. By contrast, when cells were treated with the intermediate concentration of 50  $\mu$ M, adenofection with Ad.SREBP-1c DP prevented the main part of the inhibitory effect of phenelzine on the adipose conversion process.

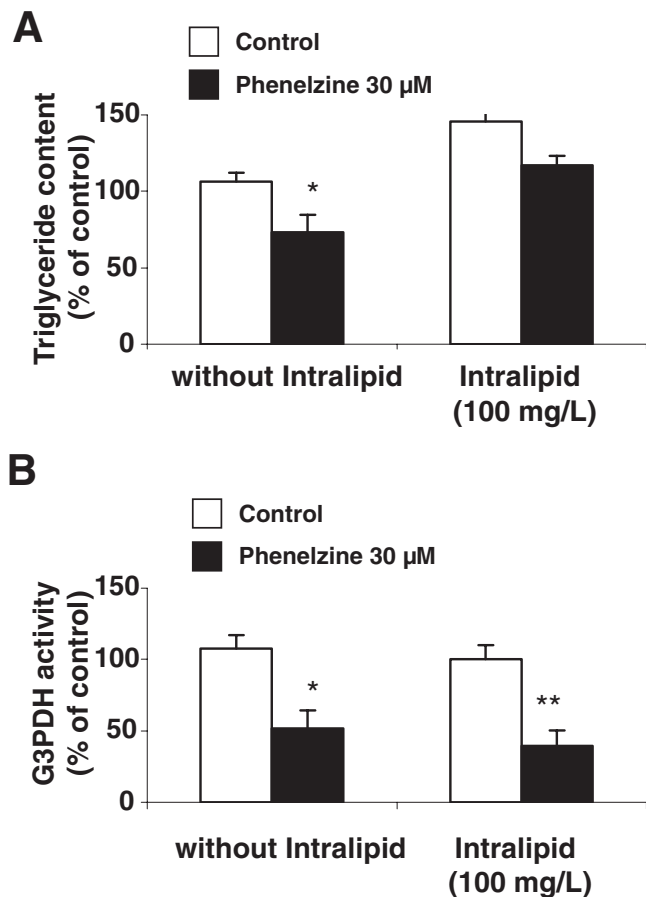
We also examined the possibility that the down-regulation of the "master" adipogenic transcription factor PPAR- $\gamma$  could exert a key role in mediating phenelzine effects. In a first set of experiments, we attempted to reverse phenelzine-induced repression of several adipocyte differentiation markers by a parallel supplementation from confluence with the PPAR- $\gamma$  agonist troglitazone. As shown in Fig. 9A, concomitant exposure of 3T3-F442A-differentiating cells to phenelzine and troglitazone did not restore the expression of ACC, C/EBP- $\alpha$ , and adiponectin proteins. This was associated with a similar pattern of morphological and biochemical parameters (data not shown). However, it was conceivable that the absence of troglitazone efficiency to reverse phenelzine action was due to the decreased expression levels of PPAR- $\gamma$  in the presence of the drug. We thus investigated in a second set of experiments the influence of a long-term phenelzine exposure on MEF-PPAR- $\gamma$  stably expressing the PPAR- $\gamma$  nuclear receptor. In these cells, adipose conversion could be amplified by

thiazolidinedione exposure, as assessed by Oil Red O staining and the induction FAS, ACC, C/EBP- $\alpha$ , and adiponectin expression in response to pioglitazone (Fig. 9B). However, even in MEF-PPAR- $\gamma$  cells, phenelzine still dramatically down-regulated adipose conversion, a phenomenon that could not be reversed by pioglitazone.

## Discussion

MAO inhibitors represent important pharmacological tools to treat severe depressions. However, these compounds are also known to exert significant side effects on weight gain and feeding behavior. Although it is generally considered that these metabolic complications are essentially related to the central effects of MAO inhibitors, it must be emphasized that these antidepressants can also inhibit MAO activity in peripheral tissues, including key players of energy balance such as liver, skeletal muscle, and adipose tissue. Moreover, several MAO inhibitors can also inhibit other amine oxidases. Although the use of phenelzine is limited to severe and resistant depression, this compound is a prototypical drug, because it potently inhibits both MAO and SSAO activities (Lizcano et al., 1996). It is thus conceivable that the metabolic side effects of several MAO inhibitors reflect both their central effects on food intake or energy expenditure and a direct targeting of peripheral tissues such as the main tank of energy stores, white adipose tissue. In our work, we document for the first time that an antidepressant of the MAO inhibitor family, phenelzine, potently alters adipose tissue development in several distinct cellular models of adipocyte differentiation. Slight differences between these cellular models exist in the potency of phenelzine to repress triacylglycerol accumulation during adipose conversion. Although T37i seem more resistant to phenelzine effect, low concentrations of the drug (1–10  $\mu$ M) already reduce cell triglyceride content in 3T3-F442A cells and primary cultures of human preadipocytes. These concentrations are compatible with therapeutic plasma phenelzine concentrations (Lichtenwalner et al., 1988, 1995; Waring and Wallace, 2007). Nevertheless, to obtain a clearcut effect, studies of the mechanisms that underlie phenelzine effects during adipogenesis were performed at high drug concentrations (30–100  $\mu$ M).

Surprisingly, although phenelzine does not influence basal lipolysis, the antidepressant inhibited rather than stimulated isoproterenol- and forskolin-stimulated lipolysis. Thus, an increased lipolytic activity was not involved in phenelzine-induced down-regulation in cell triglyceride content. Although elucidation of the mechanisms responsible for this modulation of effector-stimulated lipolysis is beyond the scope of our study, it will be of interest to determine which step(s) of the catecholamine-sensitive pathway is targeted by the drug, including hormone-sensitive lipase and adipocyte triglyceride lipase (Langin, 2006). Whatever the mechanism involved in the modulation of lipolysis by phenelzine, it cannot account for the limitation of triacylglycerol accumulation in differentiating preadipocytes. Finally, we also excluded the possibility that phenelzine could reduce basal and insulin-stimulated glucose transport, resulting in decreased substrate availability for de novo fatty acid synthesis. This finding is in agreement with previous data showing that basal and insulin-stimulated glucose transport is not altered in adipocytes obese Zucker rats receiving phenelzine or other



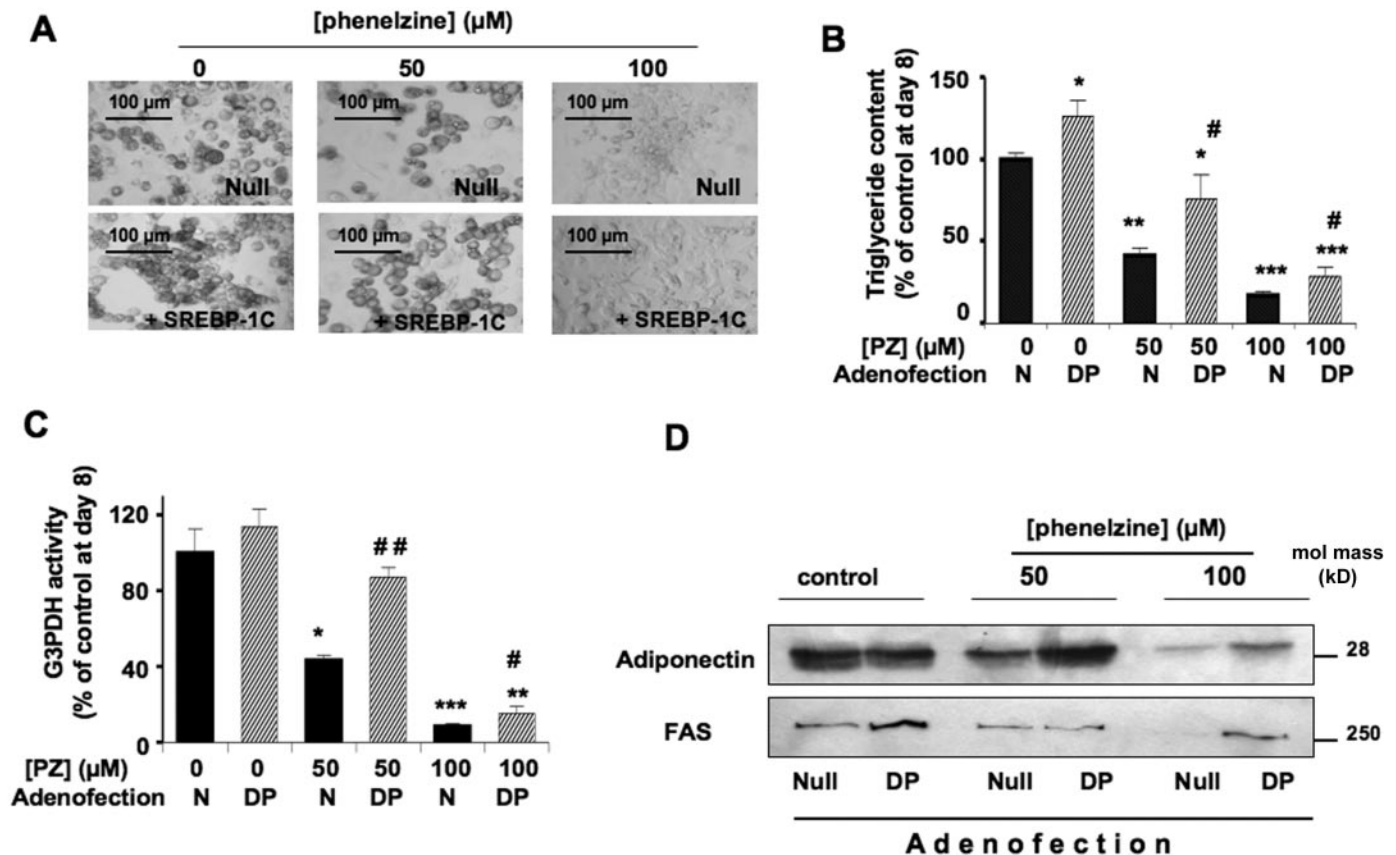
**Fig. 7.** Fatty acids prevent the inhibitory effect of phenelzine on triglyceride accumulation but not on G3PDH activity. Confluent 3T3-F442A cells (day 0) were cultured for 8 days in the absence or presence of phenelzine (30  $\mu$ M) and in the absence or presence of Intralipid (100 mg/liter). At day 8 after confluence, cell extracts were prepared and tested for triglyceride content (A) and G3PDH activity (B). Results represent the mean  $\pm$  S.E. of five to eight separate determinations and are expressed as the percentage of the control value (mean, 0.79 g/liter and 2026 nmol/min/mg for triglyceride content and G3PDH activity, respectively). \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ , phenelzine-exposed versus control cells.

amine oxydase inhibitors (Carpéné et al., 2007, 2008; Prévot et al., 2007).

Many of our experimental data converge to suggest that phenelzine markedly alters the adipocyte differentiation program and has a preferential inhibitory effect on the lipogenic pathway. Interestingly, phenelzine effects on cell triacylglycerol accumulation are detectable at lower concentrations than those required to down-regulate adipocyte differentiation, as assessed by well characterized markers of adipose conversion, aP2 or adiponectin. The concentration gap between these two biological effects, together with the exclusion of the mechanisms mentioned above, suggest that at low or intermediate phenelzine concentrations, phenelzine preferentially acts through its antilipogenic properties. In line with this, phenelzine effects on cell triglyceride stores can be completely prevented when the lipogenic pathway is bypassed by directly providing fatty acids to the cells. The potent phenelzine-induced down-regulation of SREBP-1c, the key transcription factors that drive the emergence of the lipogenic pathway, is also in agreement with a privileged alteration in de novo fatty acid synthesis from glucose. In the basal state, SREBP-1c is associated with endoplasmic reticulum. When activated, it processes to the Golgi apparatus, where it undergoes a proteolytic cleavage that allows to translocate to the nucleus to modulate the transcription

rate of its target genes. It is noteworthy that we observed that the phenelzine-induced decrease in the nuclear mature 68-kDa SREBP-1c protein was detectable at lower concentrations than those decreasing the immature high molecular mass form. Although phenelzine-induced SREBP-1c down-regulation could be the consequence of the decrease of the related mRNA levels, we cannot exclude the possibility that the antidepressant also modulates the proteolytic maturation and/or nuclear translocation of this transcription factor. Elucidation of the transcriptional or post-transcriptional mechanisms that underlie phenelzine-induced SREBP-1c decrease represents another issue. Finally, the involvement of SREBP-1c down-regulation by phenelzine in the prevention of cell triacylglycerol accumulation is also largely supported by the observation that at a submaximal drug concentration, adenovirus-driven expression of SREBP-1c restores triglyceride stores and expression of adipose conversion biochemical and molecular markers.

At the highest concentrations (30–100  $\mu$ M), phenelzine also alters the magnitude of the adipocyte differentiation process, as reflected by the decrease in the levels of the two major adipogenic transcription factors, PPAR- $\gamma$  and C/EBP- $\alpha$ . It was thus conceivable that PPAR- $\gamma$ , generally considered as the master effector of adipogenesis (Lehrke and Lazar, 2005), could play a pivotal role in the antiadipogenic effects of phenelzine. How-



**Fig. 8.** Adenoviral expression of a constitutively active SREBP-1c isoform reverses phenelzine effects on the phenotype of 3T3-F442A differentiating cells. At confluence, 3T3-F442A cells were infected either with Ad.null or with Ad.SREBP-1c DP and then immediately re-fed in a fresh differentiation medium in the absence or in the presence of 50 or 100  $\mu$ M phenelzine. Similar efficiency in viral infection was assessed by green fluorescent protein expression. Microscopic analysis (A), cell triglyceride content (B), G3PDH activity (C), and adiponectin and FAS expression (D) were analyzed at day 7 after confluence. Results represent the mean  $\pm$  S.E. of four separate determinations and are expressed as the percentage of the control value ( $0.23 \pm 0.02$  g/liter and  $2212 \pm 282$  nmol/min/mg protein for triglyceride content and G3PDH activity, respectively). \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.005$ , versus control value (Ad.null without phenelzine); #,  $p < 0.05$ ; ##,  $p < 0.005$ , phenelzine-exposed and Ad. SREBP1c DP versus phenelzine-exposed and Ad.null.



ever, two complementary approaches do not support this view. First, we show that troglitazone, a PPAR- $\gamma$ -selective agonist, does not prevent phenelzine effects on adipocyte differentiation. Although the significant residual PPAR- $\gamma$  expression after phenelzine exposure suggests that thiazolidinediones can remain efficient, troglitazone was unable to antagonize the antiadipogenic properties of the antidepressant. However, one could raise the proviso that the absence of thiazolidinedione efficiency to counteract phenelzine effects in 3T3-F442A adipocytes could be related to the lower PPAR- $\gamma$  expression levels induced by the antidepressant. Nevertheless, thiazolidinediones were also un-

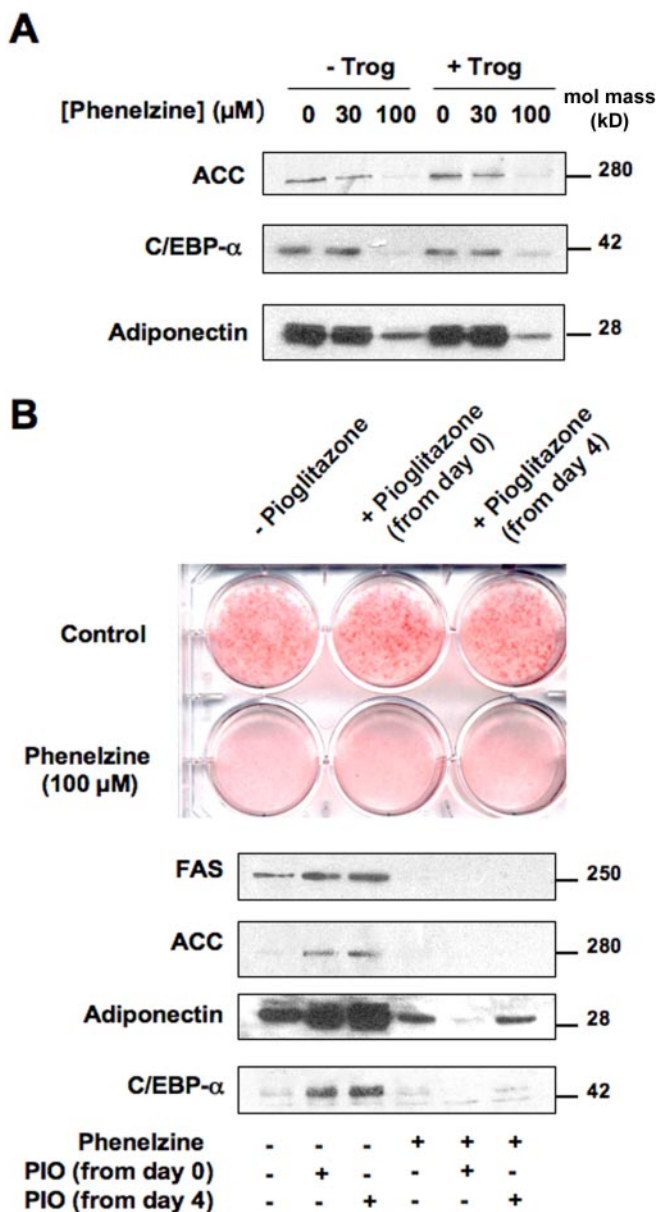
able to block the antiadipogenic effects of the drug in MEF that constitutively express PPAR- $\gamma$ . Thus, it seems unlikely that alterations in PPAR- $\gamma$  expression and/or function could represent a key mechanism that mediates phenelzine action.

Adipogenesis is associated with the induction of numerous secreted factors, named adipokines, some of which influence energy balance and insulin sensitivity. Adiponectin is thus a major adipokine with insulin-sensitizing and anti-inflammatory properties. Experimental and clinical investigations converge to demonstrate that obesity-associated adiponectin deficiency could be involved in metabolic and cardiovascular complications (Kadowaki and Yamauchi, 2005). Of interest, phenelzine strongly down-regulates adiponectin expression. It is thus tempting to speculate that modulation of adiponectin could represent an important mechanism for long-term side effects of antidepressants.

Interestingly, and in contrast to preadipocytes, mature adipocytes are insensitive to the lipid-depleting effects of phenelzine. A putative explanation for the differential efficiency of phenelzine between preadipocyte and adipocyte could also reflect differentiation-dependent variations in drug metabolism. Phenelzine is extensively metabolized primarily by oxidation via MAO. Because expression and activity of MAO is induced during adipogenesis (Bour et al., 2007), one could suggest that the degree of fat cell differentiation may influence the magnitude of cell response to phenelzine. Alternatively, one can point out that in adipocytes, phenelzine has apparent opposite effects on key mechanisms that govern homeostasis of triacylglycerol storage. On the one hand, phenelzine inhibits adipocyte differentiation and the lipogenic pathway, thus preventing cell triglyceride accumulation. On the other hand, the antidepressant reduced catecholamine- or forskolin-stimulated lipolysis, a phenomenon that promotes cell lipid engorgement. In addition, through UCP1 down-regulation, phenelzine could promote an antithermogenic effect. These findings illustrate that the drug effects on adipocytes are complex and nonunivocal. Thus, the net effect of phenelzine on adipocyte triglyceride accumulation probably involves a subtle balance between the antiadipogenic, antilipogenic, and antilipolytic effects of phenelzine at various stages of adipose conversion. It will be a major challenge to investigate *in vivo* the exact impact of phenelzine on adipose tissue development and metabolism.

Because it is generally considered that the differentiation and proliferation processes are inversely correlated, some phenelzine effects could involve an increased proliferation rate of preadipocytes. However, phenelzine did not induce cell proliferation. By extension, the development of fat mass generally observed in patients under phenelzine treatment is unlikely to be related to a direct effect of the drug to expand the population of adipocyte precursors.

Body weight gain is generally considered to be more frequent in patients treated with tricyclic antidepressants than with selective serotonin reuptake inhibitors (Zimmermann et al., 2003; Vieweg et al., 2008). MAO inhibitors, including phenelzine, are also rather associated with weight gain to an extent comparable with tricyclic antidepressants even if the contribution of increased adiposity remains unknown (Remick et al., 1989; Balon et al., 1993). This apparent discrepancy between clinical observation and our experimental data must be analyzed cautiously. As mentioned above, phenelzine effects on adipocyte metabolism are not univocal: although this compound largely prevents lipid accumulation



**Fig. 9.** PPAR- $\gamma$  is not a primary target of phenelzine-induced inhibition of adipose conversion. **A**, confluent 3T3-F442A cells were cultured in the absence or the presence of phenelzine (30 or 100  $\mu$ M) and in the absence or the presence of 10  $\mu$ M troglitazone. At day 8 after confluence, cell extracts were prepared and tested in Western blot analysis for ACC, C/EBP- $\alpha$ , and adiponectin expression. **B**, confluent MEF-PPAR- $\gamma$  cells were cultured in the absence or presence of 100  $\mu$ M phenelzine, whereas pioglitazone was added either from confluence (day 0) or from day 4 after confluence. At day 8 after confluence, cells were stained with Oil Red O, and protein extracts were prepared and tested in Western blot analysis for FAS, ACC, C/EBP- $\alpha$ , and adiponectin expression.

during the adipocyte differentiation process, it displays antilipolytic properties in mature adipocytes. In addition, regarding the wide tissue distribution of amine oxidases, phenelzine could also affect the function of other organs or tissues that have pivotal roles in the regulation of energy homeostasis. Because amines exert an essential role in the regulation of food intake (McIntyre et al., 2001), some phenelzine effects on energy homeostasis are probably mediated by targeting the central nervous system. In a more general manner, the net effect of a psychotrope on body weight and adiposity probably reflects the balance between central and peripheral effects and involves phenomena that prevent or promote energy storage. Identification of the direct peripheral effects of these compounds on liver, skeletal muscle, pancreas, and adipose tissue certainly represents a major challenge to reduce the metabolic side effects of these largely prescribed drugs.

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### References

- Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, and Vergote I (2005) Endometrial cancer. *Lancet* **366**:491–505.
- Balon R, Yeragani VK, Pohl R, Merlos B, and Sherwood P (1993) Changes in appetite and weight during the pharmacological treatment of patients with panic disorder. *Can J Psychiatry* **38**:19–22.
- Bour S, Daviaud D, Gres S, Lefort C, Prévot D, Zorzano A, Wabitsch M, Saulnier-Blache JS, Valet P, and Carpené C (2007) Adipogenesis-related increase of semicarbazide-sensitive amine oxidase and monoamine oxidase in human adipocytes. *Biochimie* **89**:916–925.
- Carpéné C, Abello V, Iffiu-Soltész Z, Mercier N, Fève B, and Valet P (2008) Limitation of adipose tissue enlargement in rats chronically treated with semicarbazide-sensitive amine oxidase and monoamine oxidase inhibitors. *Pharmacol Res* **57**:426–434.
- Carpéné C, Iffiu-Soltész Z, Bour S, Prévot D, and Valet P (2007) Reduction of fat deposition by combined inhibition of monoamine oxidases and semicarbazide-sensitive amine oxidases in obese Zucker rats. *Pharmacol Res* **56**:522–530.
- Cathala G, Savouret JF, Mendez B, West BL, Karin M, Martial JA, and Baxter JD (1983) A method for isolation of intact, translationally active ribonucleic acid. *DNA* **3**:329–335.
- Davis KE, Moldes M, and Farmer SR (2004) The forkhead transcription factor FoxC2 inhibits white adipocyte differentiation. *J Biol Chem* **279**:42453–42461.
- Després JP and Lemieux I (2006) Abdominal obesity and metabolic syndrome. *Nature* **444**:881–887.
- Dieudonné MN, Leneuve MC, Giudicelli Y, and Pecquery R (2004) Evidence for functional estrogen receptors alpha and beta in human adipose cells: regional specificities and regulation by estrogens. *Am J Physiol Cell Physiol* **286**:C655–C661.
- Enrique-Tarancón G, Castan I, Morin N, Marti L, Abella A, Camps M, Casamitjana R, Palacín M, Testar X, Degerman E, et al. (2000) Substrates of semicarbazide-sensitive amine oxidase co-operate with vanadate to stimulate tyrosine phosphorylation of insulin-receptor-substrate proteins, phosphoinositide 3-kinase activity and GLUT4 translocation in adipose cells. *Biochem J* **350**:171–180.
- Enrique-Tarancón G, Marti L, Morin N, Lizcano JM, Unzeta M, Sevilla L, Camps M, Palacín M, Testar X, Carpené C, et al. (1998) Role of semicarbazide-sensitive amine oxidase on glucose transport and GLUT4 recruitment to the cell surface in adipose cells. *J Biol Chem* **273**:8025–8032.
- Fontana E, Boucher J, Marti L, Lizcano JM, Testar X, Zorzano A, and Carpené C (2001) Amine oxidase substrates mimic several of the insulin effects on adipocyte differentiation in 3T3 F442A cells. *Biochem J* **356**:769–777.
- Foretz M, Guichard C, Ferré P, and Foulle F (1999) Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. *Proc Natl Acad Sci U S A* **96**:12737–12742.
- Kadowaki T and Yamauchi T (2005) Adiponectin and adiponectin receptors. *Endocr Rev* **26**:439–451.
- Langin D (2006) Control of fatty acid and glycerol release in adipose tissue lipolysis. *C R Biol* **329**:598–607; discussion 653–655.
- Le Lay S, Lefèvre I, Trautwein C, Dugail I, and Krief S (2002) Insulin and sterol-regulatory element-binding protein-1c (SREBP-1C) regulation of gene expression in 3T3-L1 adipocytes. Identification of CCAAT/enhancer-binding protein beta as an SREBP-1C target. *J Biol Chem* **277**:35625–35634.
- Lehrke M and Lazar MA (2005) The many faces of PPARgamma. *Cell* **123**:993–999.
- Lichtenwalner M, McMullin M, Hardy D, and Rieders F (1988) Quantitative determination of phenelzine in human fluids by gas chromatography with nitrogen specific detection. *J Anal Toxicol* **12**:98–101.
- Lichtenwalner MR, Tully RG, Cohn RD, and Pinder RD (1995) Two fatalities involving phenelzine. *J Anal Toxicol* **19**:265–266.
- Lizcano JM, Fernández de Arriba A, Tipton KF, and Unzeta M (1996) Inhibition of bovine lung semicarbazide-sensitive amine oxidase (SSAO) by some hydrazine derivatives. *Biochem Pharmacol* **52**:187–195.
- Marti L, Abella A, Carpené C, Palacín M, Testar X, and Zorzano A (2001) Combined treatment with benzylamine and low dosages of vanadate enhances glucose tolerance and reduces hyperglycemia in streptozotocin-induced diabetic rats. *Diabetes* **50**:2061–2068.
- McIntyre RS, Mancini DA, and Basile VS (2001) Mechanisms of antipsychotic-induced weight gain. *J Clin Psychiatry* **62** (Suppl 23):23–29.
- Mercier N, Moldes M, El Hadri K, and Fève B (2001) Semicarbazide-sensitive amine oxidase activation promotes adipose conversion of 3T3-L1 cells. *Biochem J* **358**:335–342.
- Mercier N, Moldes M, El Hadri K, and Fève B (2003) Regulation of semicarbazide-sensitive amine oxidase expression by tumor necrosis factor-alpha in adipocytes: functional consequences on glucose transport. *J Pharmacol Exp Ther* **304**:1197–1208.
- Moldes M, Fève B, and Pairault J (1999) Molecular cloning of a major mRNA species in murine 3T3 adipocyte lineage. differentiation-dependent expression, regulation, and identification as semicarbazide-sensitive amine oxidase. *J Biol Chem* **274**:9515–9523.
- Morin N, Lizcano JM, Fontana E, Marti L, Smih F, Rouet P, Prévot D, Zorzano A, Unzeta M, and Carpené C (2001) Semicarbazide-sensitive amine oxidase substrates stimulate glucose transport and inhibit lipolysis in human adipocytes. *J Pharmacol Exp Ther* **297**:563–572.
- Pizzinat N, Marti L, Remaury A, Leger F, Langin D, Lafontan M, Carpené C, and Parini A (1999) High expression of monoamine oxidases in human white adipose tissue: evidence for their involvement in noradrenaline clearance. *Biochem Pharmacol* **58**:1735–1742.
- Prévot D, Soltész Z, Abello V, Wanecq E, Valet P, Unzeta M, and Carpené C (2007) Prolonged treatment with aminoguanidine strongly inhibits adipocyte semicarbazide-sensitive amine oxidase and slightly reduces fat deposition in obese Zucker rats. *Pharmacol Res* **56**:70–79.
- Remick RA, Froese C, and Keller FD (1989) Common side effects associated with monoamine oxidase inhibitors. *Prog Neuropsychopharmacol Biol Psychiatry* **13**:497–504.
- Serazin-Leroy V, Morot M, de Mazancourt P, and Giudicelli Y (2000) Androgen regulation and site specificity of angiotensinogen gene expression and secretion in rat adipocytes. *Am J Physiol Endocrinol Metab* **279**:E1398–E1405.
- Teixeira M, Viengchareun S, Butlen D, Ferreira C, Cluzeaud F, Blot-Chabaud M, Lombès M, and Ferrary E (2006) Functional Isk/KvLQT1 potassium channel in a new corticosteroid-sensitive cell line derived from the inner ear. *J Biol Chem* **281**:10496–10507.
- Tong JH, D'Iorio A, and Kandaswami C (1979) On the characteristics of mitochondrial monoamine oxidase in pancreas and adipose tissues from genetically obese mice. *Can J Biochem* **57**:197–200.
- Van Gaal LF, Mertens IL, and De Block CE (2006) Mechanisms linking obesity with cardiovascular disease. *Nature* **444**:875–880.
- Vieweg WV, Levy JR, Fredrickson SK, Chipkin SR, Beatty-Brooks M, Fernandez A, Hasnain M, and Pandurangi AK (2008) Psychotropic drug considerations in depressed patients with metabolic disturbances. *Am J Med* **121**:647–655.
- Visentin V, Prévot D, Marti L, and Carpené C (2003) Inhibition of rat fat cell lipolysis by monoamine oxidase and semicarbazide-sensitive amine oxidase substrates. *Eur J Pharmacol* **466**:235–243.
- Waring WS and Wallace WA (2007) Acute myocarditis after massive phenelzine overdose. *Eur J Clin Pharmacol* **63**:1007–1009.
- Yu PH, Wang M, Fan H, Deng Y, and Gubins-Haberle D (2004) Involvement of SSAO-mediated deamination in adipose glucose transport and weight gain in obese diabetic KKAY mice. *Am J Physiol Endocrinol Metab* **286**:E634–641.
- Zennaro MC, Le Menuet D, Viengchareun S, Walker F, Ricquier D, and Lombès M (1998) Hibernoma development in transgenic mice identifies brown adipose tissue as a novel target of aldosterone action. *J Clin Invest* **101**:1254–1260.
- Zimmermann U, Kraus T, Himmerich H, Schuld A, and Pollmächer T (2003) Epidemiology, implications and mechanisms underlying drug-induced weight gain in psychiatric patients. *J Psychiatr Res* **37**:193–220.

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