

## Interleukin-15 Affects Differentiation and Apoptosis in Adipocytes: Implications in Obesity

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**Abstract** Interleukin-15 (IL-15) is an anabolic factor for skeletal muscle and several reports have described its important role as a regulator of energy homeostasis. In this study, we analyzed the effects of IL-15 on adipocyte differentiation using the 3T3-L1 preadipose cell line. The data show that IL-15 tends to reduce the rate of adipocyte proliferation, induces apoptosis, and partially stops differentiation. The signaling molecules behind these actions of the cytokine on adipose cells are: p42/p44 MAPK (which seem to be associated with the reduced rate of proliferation induced by the cytokine), STAT5 (which is related to the actions of IL-15 on differentiation), and SAPK/JNK (which are related to the increased apoptosis induced by IL-15). In conclusion, using the 3T3-L1 adipocyte cell line, the results presented here show that IL-15 exerts important effects on differentiation, proliferation and apoptosis. Altogether, the results presented here reinforce the idea that IL-15 is an important mediator that regulates adipose size and, therefore, the role of the cytokine in affecting body weight and obesity deserves additional studies.

**Keywords** IL-15 · Adipocyte · Adipogenesis · p42/44MAPK · STAT5 · SAPK/JNK

### Abbreviations

IL-15	Interleukin-15
p42/p44 MAPK	p42/44 mitogen-activated protein kinases
STAT5	Signal transducers and activator of transcription 5
SAPK/JNK	Stress-activated protein kinase/c-Jun NH2-terminal kinase
VLDL	Very low density lipoprotein
PPAR	Peroxisome/proliferator-activated receptor
WAT	White adipose tissue
JAK	Janus kinase
NFκB	Nuclear factor kappa B
AP1	Activator protein-1
PKA	Protein kinase A
ATCC	American type culture collection
MDI	Isobutylmethylxanthine, dexamethasone, insulin)
LMPCR	Ligation-mediated PCR

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### Introduction

Obesity has emerged as a pandemic in Western societies and constitutes a risk factor for many diseases such as diabetes, cancer, heart disease, and hypertension [1]. The high incidence of this pathology is the result of lifestyle habits that include overconsumption of high caloric foods and reduction of physical activity, inducing impaired

energy balance leading to constant weight gain in the form of fat [2]. Since the discovery of leptin in 1994 by Zhang and colleagues [3], the adipocyte and its products have been profoundly studied. Adipose tissue has an important and critical role in the regulation of energy homeostasis, behaving as an endocrine organ that synthesizes molecules involved in the regulation of metabolism [4]. Alterations of both the expression and activity of these molecules also contribute to the development of obesity among other pathologies [4]. IL-15 has been recently described as a cytokine that has important metabolic effects on adipose tissue [5]. IL-15 has pleiotropic functions because it exerts effects on the proliferation, survival, and differentiation of many more distinct cell types than was originally thought [6, 7]. Since the discovery of this cytokine as an anabolic factor for skeletal muscle, several reports have described its important role as a regulator of energy homeostasis [7, 8]. In-vivo studies have shown that chronic IL-15 administration (seven days) results in a 33% decrease of white adipose tissue (WAT) in healthy rats [6]. Additional studies demonstrate that the effects of the cytokine on WAT are partially caused by inhibition of both lipogenesis [9] and LPL activity [10] and by reduced lipid uptake in this tissue [5]. IL-15 also regulates liver lipogenesis, reducing the lipid content of VLDL particles [10], and thermogenesis in brown adipose tissue [11]. Some of the effects of this cytokine are mediated by upregulation of PPAR $\delta$  [5, 11] a transcription factor involved in fatty acid oxidation and other metabolic adaptations to environmental changes [12]. In summary, IL-15 could be important in the conversion of fat to lean body composition [4, 8, 10, 13].

Inhibition of adipogenesis by IL-15 in 3T3-L1 cells has been reported, suggesting a direct action of the cytokine in adipose tissue [14]. Moreover, in a study using obese rodent models, a correlation was found between the sensitivity to the fat-inhibiting effects of IL-15 and the fat expression of mRNA for signaling subunits of the IL-15 receptor in WAT [15], confirming that IL-15 has a direct effect on this tissue. Thus, it is known that the cytokine signals by activation of two different subunits also present in the IL-2 receptor, and binds specifically to the  $\alpha$  subunit of the receptor [7]. The activation of IL-15 receptor triggers different transduction pathways depending on cellular type. Thus, IL-15-mediated signaling involves proteins such as JAK/STAT, the Src family, PI3-Kinase and Akt, MAPK, NF $\kappa$ B, and AP1 in immune cells [16–19]. Regarding its signal transduction in adipose tissue, only one report has suggested that JAK and PKA act as partial mediators of the lipolytic effect that IL-15 exerts on primary pig adipocytes [20].

Taking into account the important effects of the cytokine on lipid metabolism, the objective of this study was to analyze the effects of IL-15 on adipocyte differentiation

using the 3T3-L1 preadipose cell line, and to study the signal transduction pathways mediating the effects of the cytokine.

## Materials and Methods

### Cell Culture and Adipocyte Differentiation

3T3-L1 preadipocytes (ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM) with high glucose (Invitrogen) supplemented with 10% (*v/v*) fetal calf serum (Invitrogen), 25 mM HEPES pH 7.0, 1,000 U/mL penicillin, 1,000 U/mL streptomycin, and 25  $\mu$ g/mL fungizone (BioWhittaker). To induce adipocyte differentiation, cells were grown for 2 days postconfluence and then treated for 2 days with differentiation medium of DMEM with high glucose supplemented with 10% (*v/v*) fetal bovine serum (Invitrogen), 25 mM HEPES pH 7.0, 1,000 U/mL penicillin, 1,000 U/mL streptomycin, and 25  $\mu$ g/mL fungizone, plus MDI (0.5 mM isobutylmethylxanthine, 0.25  $\mu$ M dexamethasone and 5  $\mu$ g/mL insulin). On day 2 the cells were re-fed with differentiation medium that contained insulin (5  $\mu$ g/mL) instead of MDI, and every 2 days thereafter with differentiation medium alone. The cells were completely differentiated 14 days after induction. 3T3-L1 cells were treated with 10 ng/mL IL-15 (Peprotech) or with PBS for 7 days before inducing differentiation (referred to hereafter as preadipocytes), for 7 days after inducing differentiation (referred to as 7d-adipocytes), and for 7 days after they were differentiated (referred to as 14d-adipocytes). All experimental groups were analyzed at the end of the treatment.

### Protein Extraction and Western Blotting

For protein extraction the cells were scraped into ice-cold buffer containing 10 mM Tris pH 7.5, 10 mM EDTA, 400 mM NaCl, 10% glycerol, 0.5% Nonidet P-40, 5 mM NaF, and 0.01 mM sodium orthovanadate, in the presence of protease inhibitors, and then sonicated for 10 s. The samples were then centrifuged at 13,000 rpm for 10 min and the supernatants were collected and protein concentration was determined by use of the BCA method (Pierce, USA). Equal amounts of protein (50  $\mu$ g) were heat-denatured in sample-loading buffer (50 mM Tris-HCl, pH 6.8, 100 mM DTT, 2% SDS, 0.1% bromophenol blue, 10% glycerol), resolved by SDS-polyacrylamide gel electrophoresis (10% polyacrylamide, 0.1% SDS) and transferred to Immobilon membranes (Immobilon PVDF, Millipore, USA). The filters were blocked with 5% PBS-non-fat dried milk or 5% BSA and then incubated with polyclonal antibodies: anti-phosphoAKT,

anti-phosphoSAPK/JNK, anti-phosphoJAK1, anti-JAK1, anti-phosphoSTAT5, anti-STAT5 (Cell Signaling Technology, Beverly, USA), anti-AKT, anti-SAPK/JNK1, anti-phosphop38 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and anti-p38 (Calbiochem, Germany). An anti-tubulin polyclonal antibody (Santa Cruz Biotechnology) was used as a control for the different studies. Donkey anti-mouse peroxidase-conjugated IgG (Jackson ImmunoResearch, West Grove, USA), and goat anti-rabbit horseradish peroxidase conjugate (Bio-Rad, USA) were used as secondary antibodies. The membrane-bound immune complexes were detected by use of an enhanced chemiluminescence system (EZ-ECL, Biological Industries, Israel).

#### Cell-Proliferation Studies

3T3-L1 cells were seeded in multiwells plates and cultured for 24 h for cell attachment. The cells were then serum-starved for 24 h and treated with 10 ng/mL IL-15 or vehicle solution depending on the experimental group. After 24 h of treatment, 1  $\mu$ Ci methyl- $^3$ H]thymidine (Amersham) was added to each well, and cells were incubated for 24 h for thymidine incorporation into DNA [21, 22]. For measuring the cell-proliferation rate, cells were washed twice with PBS, and DNA was precipitated by use of trichloroacetic acid. Finally, cells were homogenized and the total amount of lysate was dissolved in 4 mL liquid-scintillation fluid for total radioactivity estimation in a liquid scintillation counter.

#### DNA Extraction and Apoptosis

The studies were carried out on 14d-adipocytes. For DNA extraction, cells were resuspended in Kauffman buffer (0.5 M Tris, 2 mM EDTA, 10 mM NaCl, and 1% SDS) and incubated for 12 h in the presence of 200  $\mu$ g/ml proteinase K. DNA was extracted with phenol–chloroform [23] and apoptosis was measured by LMPCR (ligation-mediated PCR) [24], using the  $\beta$ -actin gene as control. The PCR samples were checked by 1.5% agarose gel electrophoresis and stained with ethidium bromide. The percentage of DNA fragmentation was quantified by scanning densitometry (Phoretix International, Newcastle upon Tyne, UK).

#### Oil Red O Staining

In order to assess the stage of differentiation, cells were stained by oil red O dye, lipid content being determined in both 7d-adipocytes and 14d-adipocytes. The cells were fixed with 3% paraformaldehyde and stained with the lipophilic dye oil red O (Sigma) dissolved in isopropanol, as previously described [14]. Stained cells were visualized

by bright-field microscopy and photographed. Diameter of lipid droplets was measured by bright-field microscopy at  $\times 40$ .

#### Annexin V–Propidium Iodide Staining

The staining was performed on 14d-adipocytes. To determine apoptosis by the annexin–propidium iodide method, cells were seeded on coverslips. After 3T3-L1 differentiation, cells were serum-starved for 24 h followed by 24 h-treatment with IL-15 (100 ng/mL). For annexin V–propidium iodide determination, the cells were fixed and stained by use of a commercial kit (rhAnnexin V/FITC/propidium iodide; Bendermedsystems, Pharmingen, San Diego, CA, USA). Apoptosis was assessed by laser-scanning microscopy with a laser wavelength of 495 nm.

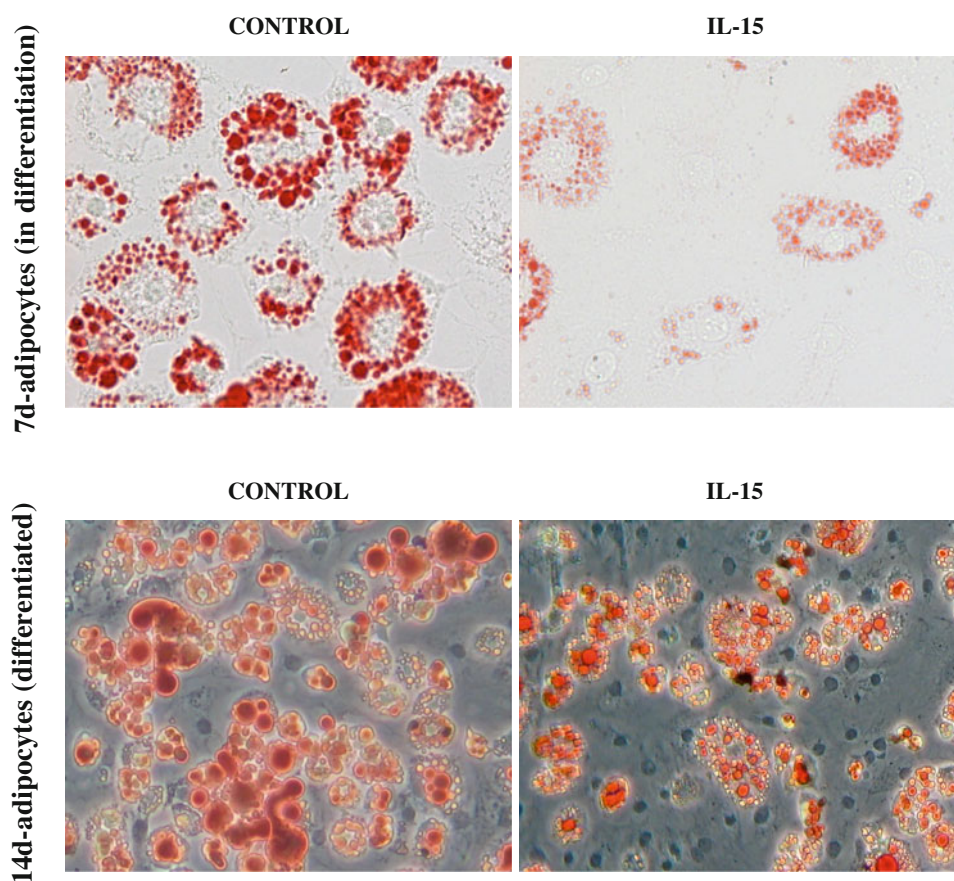
#### DAPI Staining

DAPI staining was carried out using 14d-adipocytes. For analysis of nuclei condensation, the cells were seeded on coverslips. Twenty-four hours after serum deprivation, cells were treated for 24 h with 100 ng/mL IL-15. The cells were then washed with ice-cold PBS, fixed with 70% ethanol for 10 min, stained with a DAPI solution (1  $\mu$ g/mL) for 20 min, then washed twice and mounted on glass slides with Vectashield<sup>®</sup> Mounting Medium; Vector Laboratoires H-1000, Southfield, MI, USA). DAPI staining was analyzed by use of an epifluorescence microscope (Leica, Solms, Germany).

#### Real-Time PCR (Polymerase Chain Reaction)

First-strand cDNA was synthesized from total RNA with oligo dT15 primers and random primers pdN6 by using a cDNA synthesis kit (Transcriptor Reverse Transcriptase, Roche, Barcelona, Spain). Analysis of mRNA levels for the genes from the different proteolytic systems was performed with primers designed to detect the following gene products: PPAR $\delta$  (5' GAAGCCATCCAGGACACCAT 3'; 3' AAGG TCTCACTCTCCGTCTT 5'), leptin (5' TTCACA CACGCAGTCGGTAT 3'; 3' CATTCA GGGCTAAGGTC CAA 5') and 18S (5' GCGAATGGCTCATTAAAT CAGTTA 3'; 3' GC ACGTAAATAGTCTAGTTTTGGT 5'). To avoid the detection of possible contamination by genomic DNA, primers were designed in different exons. The real-time PCR was performed using a commercial kit (LightCycler<sup>™</sup> FastStart DNA Master<sup>PLUS</sup> SYBR Green I, Roche, Barcelona, Spain). The relative amount of all mRNA was calculated by using the comparative  $C_T$  method. The invariant control for all studies was 18S mRNA.

**Fig. 1** Oil red O staining of both 7d-adipocytes (in differentiation) and 14d-adipocytes (completely differentiated) after treatment with vehicle or IL-15. Oil red O dye stains lipid content of the cells. These are representative photographs from six independent experiments. The results are means  $\pm$  SEM for 100 cells and are expressed in  $\mu\text{m}$  (diameter of lipid droplets measured by bright-field microscopy at  $\times 40$ )



### Statistical Analysis

All data were analyzed by use of Student's *t* test. Statistical significance of results is indicated by: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

### Results

To check the degree of adipocyte differentiation, cells were stained with oil red O dye to estimate lipid content (Fig. 1). It can be seen that cells were clearly differentiated and how IL-15 resulted in a significant decrease in lipid content. Levels of different markers (PPAR- $\gamma$ 2 and leptin) also indicated a clear effect of IL-15 on lipid content (Table 1). Because p42/44 MAPK play a central role in the regulation of cell proliferation and cell survival [25–27], their content was analyzed. In preadipocytes, IL-15 treatment caused a significant decrease (60% less than control) of both the phosphorylated form and the total amount of p42/44 MAPK (Fig. 2a) with no differences in the ratio (Fig. 2b). However, IL-15 treatment of early differentiated adipocytes (7 days) caused a strong decrease of the p44 MAPK protein content (84% less than in the control) (Fig. 2a, b). On the other hand, in 14d-adipocytes, IL-15 treatment led

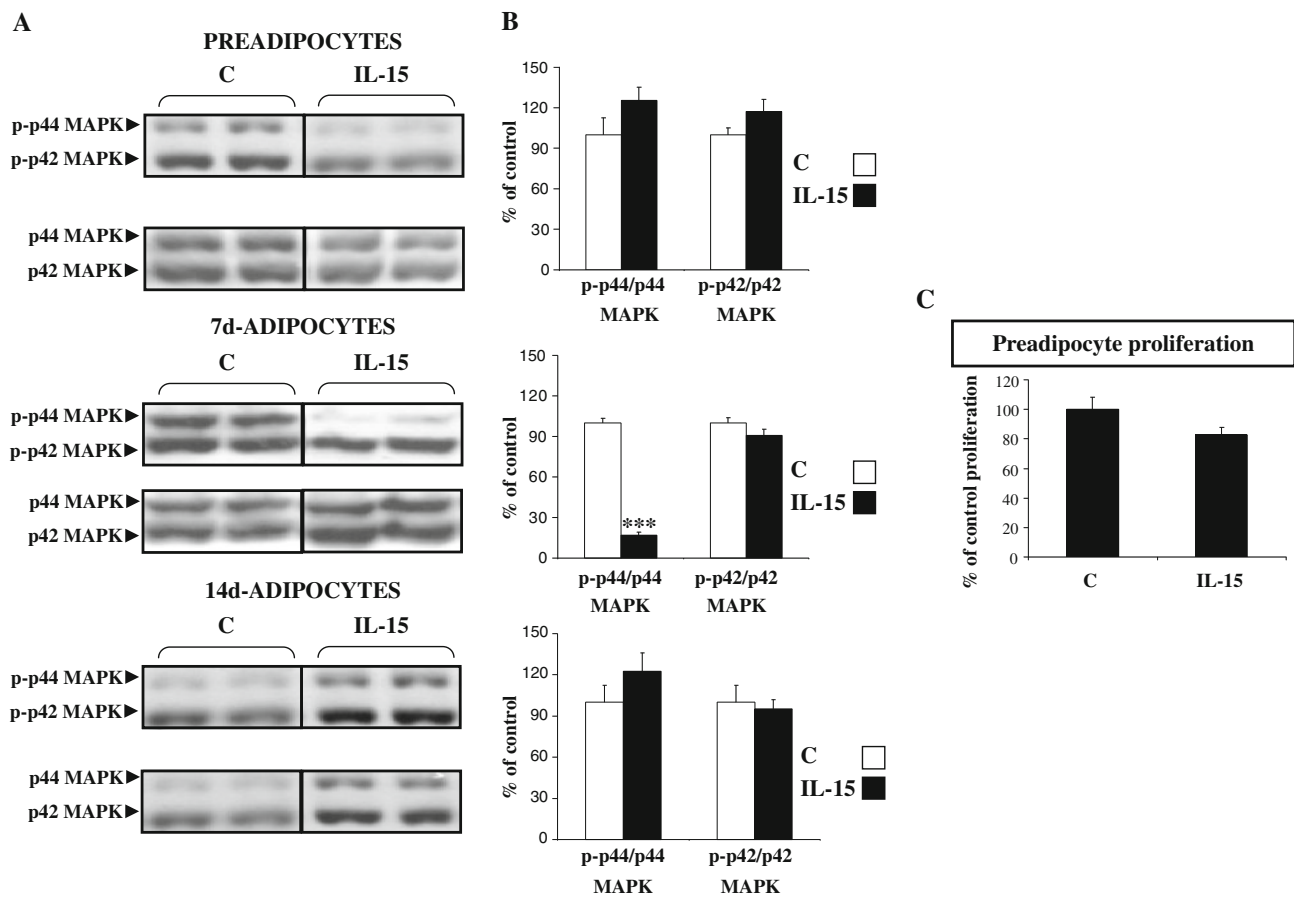
**Table 1** Effects of IL-15 on 7d-adipocytes' mRNA content of the differentiation markers

Experimental group	Control	IL-15
PPAR $\gamma$	100 $\pm$ 48 (5)	26 $\pm$ 11 (4)
Leptin	100 $\pm$ 30 (5)	17 $\pm$ 8 (4)*

For more details see the “Materials and Methods” section. Results are mean  $\pm$  SEM (*n*) for the number indicated in parentheses. The results are expressed as a percentage of controls. Values that are significantly different by the Student's *t* test from the control group are indicated by \* $p < 0.05$

to an increase of both forms of p42/44 MAPK (phosphorylated active p44 73% and p42 29%; total amount p44 42% and p42 47%) protein (Fig. 2a).

To relate the effects of the cytokine to cell differentiation, the JAK/STAT pathways were examined. The total and activated amount of both JAK and STAT proteins in the different stages of adipocyte differentiation under IL-15 treatment were determined. Despite a significant increase in the total amount of JAK1 and STAT5 proteins in 3T3-L1 preadipocytes (25 and 80% respectively) (Fig. 3a), the ratio between the active or phosphorylated form and the total protein content indicated that there was a significant decrease of active JAK1 protein whereas no significant changes were observed for STAT5 (Fig. 3b). During the



**Fig. 2** Effects of IL-15 on MAPK protein content, cell cycle distribution, and proliferation rate. *C* control, *IL-15* IL-15-treated cells. **a** Representative Western blot pattern of both phosphorylated and total p42/p44 MAPK. The images were densitometrically analyzed and corrected for tubulin levels as loading control (images not shown). Data were then statistically analyzed (Student's *t* test). **b** Ratio of densitometric analysis of phosphorylated and total form of

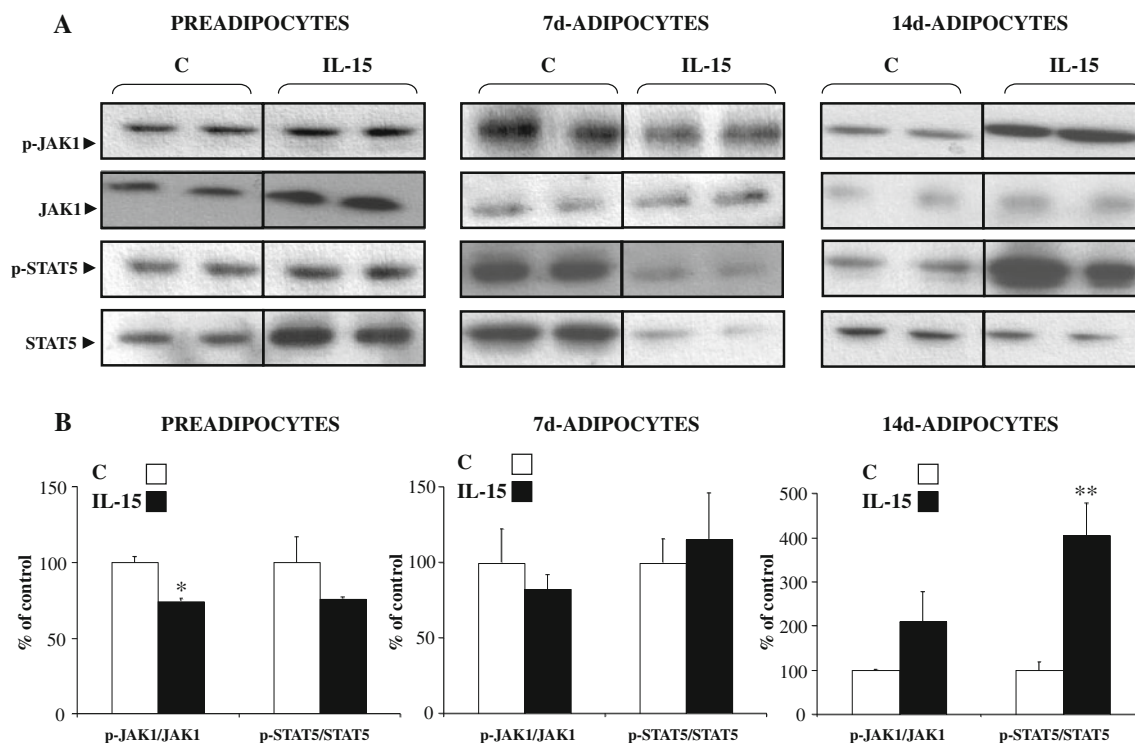
p42/p44 MAPK content. The results are means  $\pm$  SEM for 4 or 5 samples and are expressed as percentages of control values. Values that are significantly different by the Student's *t* test from the control group are indicated by \*\*\* $p < 0.001$ . **c** Effects of IL-15 on proliferation rate of preadipocytes expressed as a percentage of proliferation of control cells

differentiation of 3T3-L1 cells (d7-adipocytes), cytokine treatment induced a decrease in both the active form and the total amount of STAT5 protein, despite no changes were observed in the ratio (Fig. 3a, b). However, in d14-adipocytes, the IL-15 treatment resulted in a significant increase of both JAK1 and STAT5 phosphorylated proteins (127 and 300% respectively) (Fig. 3a) also in the ratio (Fig. 3b), suggesting activation of the JAK/STAT pathway in mature 3T3-L1 adipocytes.

AKT is another important signaling protein for cell proliferation and differentiation [28]. No differences were observed in AKT protein content in 3T3-L1 preadipocytes treated with IL-15 (Fig. 4a). However, treatment with this cytokine induced a significant increase in the total amount of AKT protein (42%) (Fig. 4a) and reduced the ratio (Fig. 4b), therefore modulating the activity of this pathway. Finally, IL-15 caused an increase in both activated and

total of AKT protein in 14d-adipocytes (70 and 54%, respectively) (Fig. 4a).

p38 and SAPK/JNK belong to the MAPK signaling pathway and are known to be involved in the regulation of differentiation and cell apoptosis. Interestingly, these proteins can regulate each other [25, 29–32]. In preadipocytes, IL-15 treatment caused a decrease in the total amount of p38 (47%) without affecting its activated phosphorylated form, as shown in Fig. 5a, b. Moreover, in 7d-adipocytes IL-15 treatment also caused a significant reduction in the total amount of p38 (34%), leading to a significant increase of the ratio phospho-p38/p38 (Fig. 5a, b). Finally, in 14d-adipocytes the treatment resulted in a significant decrease in the percentage of activation, but an increase in the total amount of p38 protein (73%). In order to see the effects of IL-15 on the SAPK/JNK pathway, the two proteins that form the dimer (SAPK/JNK54/46) were analyzed. IL-15



**Fig. 3** Effects of IL-15 on JAK1 and STAT5 protein content. *C* control, *IL-15* IL-15-treated cells. **a** Representative Western blot pattern of both phosphorylated and total JAK1 and STAT5. The images were densitometrically analyzed and corrected for tubulin levels as loading control (images not shown). Data were then statistically analyzed (Student's *t* test). **b** Ratio of densitometric

analysis of phosphorylated and total form of JAK1 and STAT5 content. The results are means  $\pm$  SEM for 4 or 5 samples and are expressed as percentages of control values. Values that are significantly different by the Student's *t* test from the control group are indicated by \**p* < 0.05, \*\**p* < 0.01

treatment caused a significant decrease of the ratio between phospho-SAPK/JNK and SAPK/JNK total (in both JNK54 and 46) in preadipocytes (Fig. 5b). Interestingly, during adipocyte differentiation (14d-adipocytes), the ratio between the active and total forms of these proteins was increased (Fig. 5b).

It is important to note that the increase in p38 and SAPK/JNK in mature 14d-adipocytes has been related to increased apoptosis [25, 31]. Thus, to determine if this could be a satisfactory explanation for the observed increase in the p38 protein, apoptosis was studied by the LMPCR method. Figure 6a shows a clear and significant increase of the DNA ladder levels after IL-15 treatment in 14d-adipocytes, supporting increased apoptosis as a result of the cytokine treatment. DAPI and annexin-propidium iodide analysis confirmed the effects of IL-15 on apoptosis (Fig. 6b, c).

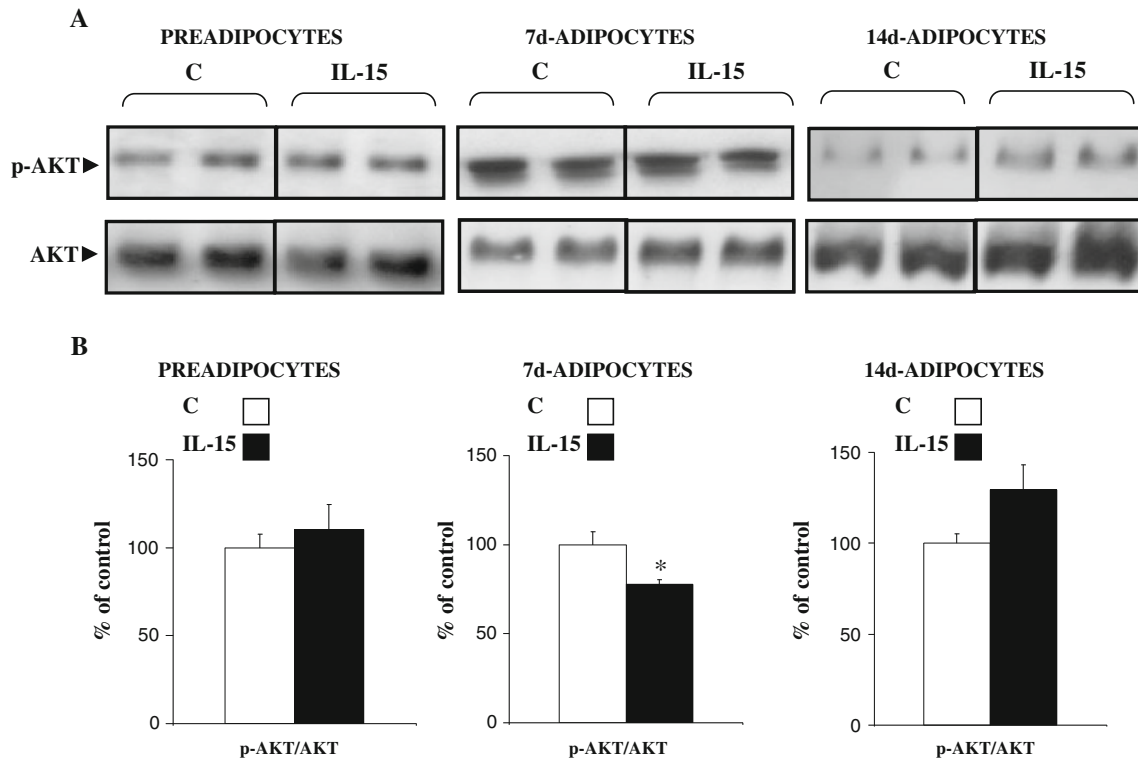
## Discussion

The accretion of WAT observed in obese patients can be because of an increase in cell size, cell number or both [33], indicating that the molecular mechanisms that

regulate preadipose cell growth, adipose differentiation, and lipogenesis in fat cells, have a key role in controlling the energy balance [33]. Cytokines seem to be major regulators of adipose tissue metabolism [34]. Taking this into consideration, IL-15 is a cytokine of special interest because it causes an important reduction of WAT weight by altering several important processes within this tissue, for example lipogenesis [9, 15], LPL activity, or lipid uptake [5, 10]. Furthermore, it is important to note that our results from oil red O staining confirm that IL-15 is able to inhibit adipogenesis in 3T3-L1 cells as previously described [14].

IL-15 signaling pathways have been widely studied in the immune system since this cytokine was firstly described in immune cells in 1994 [35, 36]. However, very few investigations have been focused on the proteins involved in IL-15 signal transduction in adipose tissue or other non-immune system tissues. Ajuwon and Spurlock [20] described the direct regulation of lipolysis by IL-15 in primary pig adipocytes through a pathway regulated in part by PKA and JAK proteins.

In order to study the signaling of IL-15 on adipocytes, we chose the 3T3-L1 cell line. This is an immortal fibroblast line derived from nonclonal Swiss 3T3 cells that are



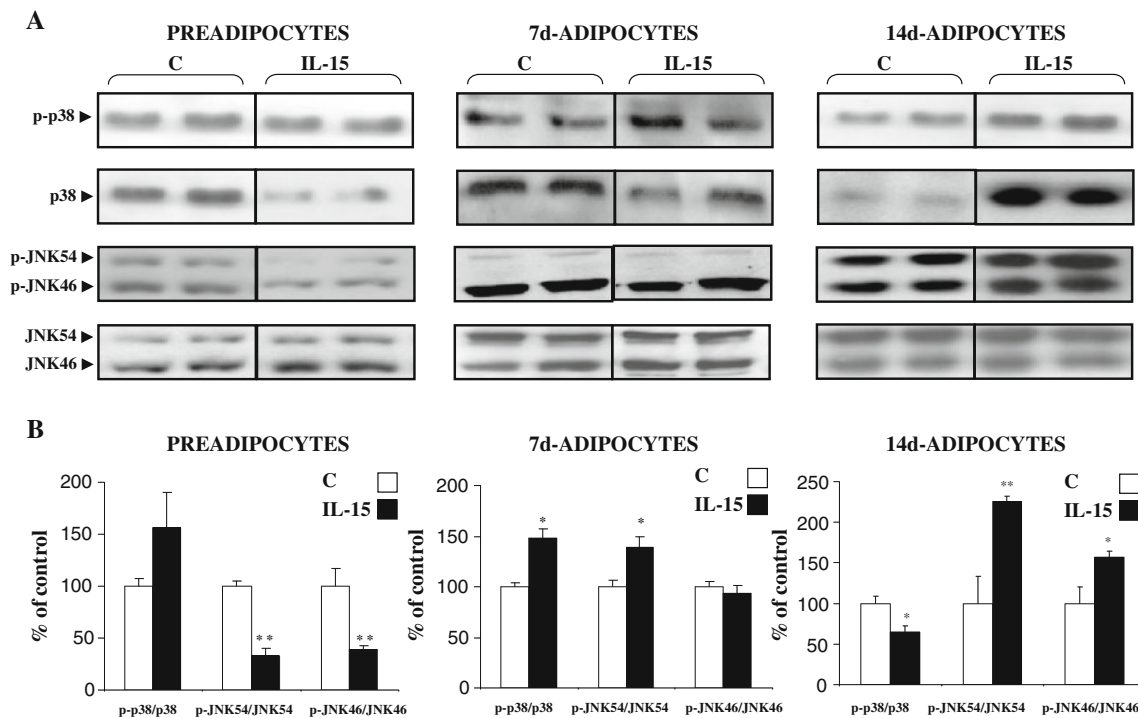
**Fig. 4** Effects of IL-15 on AKT protein content. *C* control, *IL-15* IL-15-treated cells. **a** Representative Western blot pattern of both phosphorylated and total AKT. The images were densitometrically analyzed and corrected for tubulin levels as loading control (images not shown). Data were then statistically analyzed (Student's *t* test).

**b** Ratio of densitometric analysis of phosphorylated and total form of AKT content. The results are means  $\pm$  SEM for 4 or 5 samples and are expressed as percentages of control values. Values that are significantly different by the Student's *t* test from the control group are indicated by \**p* < 0.05

already committed to the adipocytic lineage [37]. This cellular model has some limitations, because the adipose tissue matrix has an important role in adipocyte differentiation. However, when these cells receive the appropriate hormonal inducers, they undergo differentiation to mature fat cells [37–39]. In fact, the first stage before differentiation is growth arrest, which is achieved in cultured cells by contact inhibition (named in this study as the preadipocyte group). When the cultured cells receive the differentiation cocktail (isobutylmethylxanthine, dexamethasone, and insulin), they begin the next step of differentiation, called clonal expansion. This is followed by a second growth arrest which is associated with several changes in protein expression. After this, the cells acquire a round morphology, accumulate lipid, become insulin-sensitive, and express typical gene markers (for example PPAR $\gamma$ ) of completely differentiated adipocytes [40].

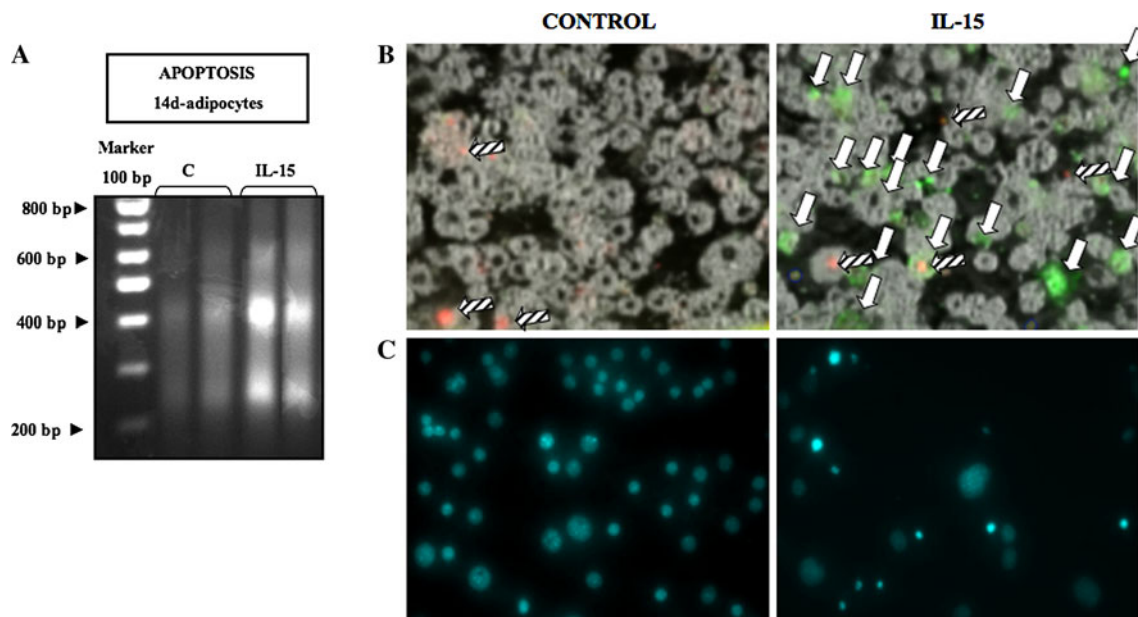
In immune cells, IL-15 signals through several molecules, for example MAPK, AKT, or JAK/STAT. Among these, p42/44 MAPK is highly related to the promotion of cell division [27] and this could be the role of this protein in 3T3-L1 preadipocytes. Indeed, IL-15 seems to downregulate p42/44 MAPK levels in preadipocytes, and it could lead to a tendency of a lower proliferation rate.

The JAK/STAT pathway is a predominant signaling cascade for cytokines including the IL-15 signaling pathway [7]. In addition, it has been described that during adipocyte differentiation, expression levels of STAT5 are highly induced and dysregulation of this cell process attenuates induction of this protein [41]. Floyd and Stephens [42] stated that STAT5A ectopic expression in nonprecursor cells led to fat cell differentiation, confirming the importance of this signaling protein in this adipocyte process. As mentioned in the “Results” section, IL-15 treatment resulted in a decrease in differentiation markers in 7d-adipocytes. Interestingly, treatment of 7d-adipocytes with IL-15 clearly resulted in downregulation of the levels of STAT5 protein, indicating that the effects of IL-15 on adipogenesis could be partly mediated through STAT5 protein. Conversely, in mature adipocytes STAT5 protein has been reported to have an antilipogenic role by inhibiting expression of fatty acid synthase, the central enzyme for de-novo lipogenesis [43]. Furthermore, it has been reported that IL-15 induces loss of adipose tissue partly by inhibiting lipogenesis [9]. Indeed, IL-15 treatment of 14d-adipocytes results in a significant increase in the activity of the JAK/STAT pathway and a decrease in lipid content, suggesting that this cytokine



**Fig. 5** Effects of IL-15 on p38 and SAPK/JNK protein content. *C* control, *IL-15* IL-15-treated cells. **a** Representative Western blot pattern of both phosphorylated and total p38 and SAPK/JNK. The images were densitometrically analyzed and corrected for tubulin levels as loading control (images not shown). Data were then statistically analyzed (Student's *t* test). **b** Ratio of densitometric

analysis of phosphorylated and total form of SAPK/JNK content. The results are means  $\pm$  SEM for 4 or 5 samples and are expressed as percentages of control values. Values that are significantly different by the Student's *t* test from the control group are indicated by \**p* < 0.05, \*\**p* < 0.01



**Fig. 6** Effects of IL-15 on apoptosis. *C* control, *IL-15* IL-15-treated cells. **a** DNA fragmentation analysis of adipocytes was assessed by the LMPCR method. **b** Annexin V–propidium iodide stained 3T3-L1 cells observed by laser-scanning microscopy. Cells were detected negative for Annexin V-FITC and PI (viable, or no measurable apoptosis),

positive for Annexin V-FITC and negative for PI (green-colored and white arrows, early apoptosis, membrane integrity is present), and, finally, Annexin V-FITC and PI positive (red-colored and hatched arrows, end-stage apoptosis and death). **c** DAPI stained 3T3-L1 cells observed by epifluorescence microscopy (color figure online)



could also act through these proteins to control adipocyte lipogenesis.

There are contradictory reports on the role of p42/44 MAPK in adipogenesis. On the one hand, some studies have implicated these proteins in the differentiation process [44] and, on the other hand, Font de Mora et al. [45] stated that they are not necessary for this process but rather these proteins exert an antagonizing effect on adipogenesis. However, it is important to note that after insulin treatment—as a differentiation stimulus—the MAPK and AKT pathways are activated [46]. Insulin treatment seems to exert a dual effect on 7d-adipocytes, by inducing mitogenesis in growing cells, and differentiation in confluent cells through p42/44 MAPK and AKT respectively. In our study, the observation that IL-15 inhibited p44 MAPK reinforces the role of this protein in the differentiation process. On the other hand, the activation of p42/44MAPK by IL-15 at day 14 could also be correlated with cytokine blockage of the differentiation process, because p42/44MAPK has been involved in inhibition of terminal adipose conversion. Interestingly, sustained activation of p42/44 MAPK has been demonstrated to inhibit terminal adipose conversion by inhibition of PPAR $\gamma$ , at least [47]. IL-15 could act by blocking the final stages of adipogenesis in 14d-adipocytes by increasing the protein level of p42/44 MAPK and consequently could be blocking PPAR $\gamma$  activity. Additionally, and because the AKT pathway has a key role in adipogenesis, it has been reported that constitutive activation of AKT induces spontaneous adipogenesis in 3T3-L1 cells [28]. The IL-15-induced decrease of the activation ratio at day 7 supports the effects of this cytokine on adipogenesis.

Although previous work established a direct relationship between p38 upregulation and apoptosis in 3T3-L1 adipocytes [29], the results of our study showed upregulation of total p38 in mature adipocytes without affecting the activated protein. In addition, in 14d-adipocytes IL-15 treatment caused a significant increase in SAPK/JNK, which seems to lead to an increment in apoptosis levels as shown in the apoptotic assays (Fig. 6). These results agree with the study of Yang et al. [31], who reported that increased levels of SAPK/JNK are also directly related to apoptosis.

In conclusion, this study expands knowledge of the actions of IL-15 on body fat [8, 10] and confirms the anti-adipose tissue actions of the cytokine. IL-15 could, thus, regulate energy homeostasis by affecting adipose tissue in several cell processes including proliferation, differentiation, lipogenesis, and apoptosis. These actions on adipocytes may be mediated by different signaling pathways: p42/44 MAPK in proliferation and final conversion to adipocytes, STAT5 during differentiation process and lipogenesis, and SAPK/JNK in apoptosis in mature

adipocytes. Additional studies are needed to further understand the role of IL-15 in adipogenesis.

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**Conflict of interest** All authors of this research have no conflict of interest related with employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

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