Endocrine Journal 2008, **55** (5), 827–837

Effects of Leptin on Lipid Metabolism and Gene Expression of Differentiation-Associated Growth Factors and Transcription Factors during Differentiation and Maturation of 3T3-L1 Preadipocytes

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Abstract. The present study was designed to determine the effects of leptin on lipid metabolism and gene expression during differentiation and maturation of the 3T3-L1 murine preadipocyte. The preadipocytes were induced to differentiate in a growth medium containing 10% calf serum and a hormonal cocktail for 2 days. The cells were next allowed to maturate for 14 days in the growth medium supplemented with 10 μg/ml insulin or 500 ng/ml insulin-like growth factor (IGF)-I in the absence or presence of supplemented leptin. Leptin, at a dose of 5 to 500 ng/ml, had no effect on proliferation of undifferentiated 3T3-L1 cells. However, leptin suppressed the insulin- or IGF-I-stimulated lipid accumulation and enhanced the release of glycerol, a measure of lipolysis, in a dose-dependent manner during and after the maturation of the cell. Moreover, leptin at a dose of 50 ng/ml inhibited IGF-I gene expression during the entire differentiation and maturation and also peroxisome proliferator activated receptor (PPAR)-γ expression during late maturation as monitored by semi-quantitative reverse transcription-polymerase chain reaction. However, leptin exerted no effect on the expression of transforming growth factor-β, CCAT/enhancer binding protein-α and PPAR-δ. Taken together, results suggest the anti-lipogenic and lipolytic effects of leptin in differentiating and mature adipocytes may have been partly mediated by suppressing the expression of PPAR-γ and IGF-I genes.

Key words: Leptin, IGF-I, PPAR, Adipocyte, Differentiation, Lipid

(*Endocrine Journal* **55**: 827–837, 2008)

ADIPOCYTES are highly specialized cells which play an important role in energy homeostasis by harboring energy reservoirs as lipid droplets consisting of triglycerides [1, 2]. These reservoirs, however, have been implicated in a host of major human health problems, because an excessive or insufficient energy reserve results in a metabolic disorder known as obesity or lipodystrophy, respectively [3, 4]. The cellular development and subsequent metabolic processes controlling the energy reserve of adipocdytes are regulated by a number of transcription factors and autocrine/paracrine as well as endocrine agents. Insulin-like growth factor (IGF)-I stimulates the proliferation and differentiation including lipid synthesis and also inhibits lipolysis in the adipose cell lines in a fashion similar to that of insulin [5–7]. Transforming growth factor-β also has a stimulatory effect on proliferation of preadipocytes [8], but, unlike IGF-I, this peptide inhibits differentiation of preadipocyte cell lines [9,

Received: April 15, 2008
Accepted: April 16, 2008
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10]. These two growth factors are thus essential regulators in adipocyte proliferation and differentiation. Adipocyte development is also regulated by a number of CCAT/enhancer binding protein (C/EBP)- and peroxisome proliferator activated receptor (PPAR)-family transcription factors [2, 11–13]. In this regard, PPAR-δ, C/EBP-β and -δ are expressed at the onset of preadipocyte differentiation [14, 15], whereas expression of PPAR-γ and C/EBP-α, which trigger the expression of adipocyte-specific genes, is induced during terminal differentiation of the adipocyte lineage [16–18].

Leptin is an established endocrine as well as autocrine/paracrine hormone secreted from adipocytes which plays an important role in regulating the food intake and peripheral energy balance [19, 20]. However, little is known about the role of leptin as a potential metabolic regulator in differentiating adipocytes, although this 16-kDa peptide is known to be expressed during adipocyte differentiation concomitantly with the expression of adipocyte-specific genes [21]. The present study was therefore undertaken to examine the effects of leptin on lipid metabolism and gene expression related to cellular development during differentiation and maturation of 3T3-L1 preadipocytes and thereby to find insights into the role of this peptide as a potential autorcinre/paracrine regulator in adipocyte development.

Materials and Methods

Determination of the mitotic activity of the preadipocytes by MTT assay

The 3T3-L1 preadipocytes of a mouse embryo fibroblast origin (ATCC, Rockville, MD, USA) were cultured in a growth medium [high-glucose Dulbeco's Modified Eagle's Medium (DMEM) containing 100 unit/ml penicillin, 100 μg/ml streptomycin (all of these from Gibco-Invitrogen, Carlsbad, CA, USA) and 10% calf serum (Sigma Chemical Co., Saint Louis, MO, USA) in 100-mm culture dishes to confluence at 37°C in a humidified atmosphere with 5% CO_2. After washing twice with PBS and harvesting, the preadipocytes were seeded in 96-well plates (BD-Falcon Co., San Jose, CA, USA) at a density of 1.0×10^4 cells per well and incubated in 0.2-ml growth medium for 24 hr. Cells were refed with the growth medium containing 0, 5, 50, or 500 ng/ml recombinant mouse leptin (R&D Systems, Minneapolis, MN, USA) and further incubated for zero to six days.

Mitotic activity of the preadipocytes was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5,-diphenyl tetrazolium bromide (MTT; Sigma Chemical Co.) method as described by Scudiero *et al.* [22]. Briefly, following addition of 0.1 ml of 2 mg/ml MTT to 0- to 6-day cultured preadipocytes, the culture plate was incubated for 2 hr at 37°C. The MTT-treated culture medium was removed, after which each well received 0.15-ml dimethyl sulfoxide and spectrophotometry at 595 nm on an enzyme-linked immunosorbent assay plate reader.

Induction of differentiation of preadipocytes

The 3T3-L1 preadipocytes were induced to differentiate according to the procedure of Student *et al.* [23], with a few modifications [24, 25]. The harvested cells, which had been cultured to confluence in the growth medium, were incubated for 2 days (day –2 to 0) in the growth medium containing MDI (0.5 mM methylisobutylxanthine, 1 μM dexamethasone and 10 μg/ml insulin; all reagents from Sigma Chemical Co.) to initiate the differentiation. At day 0, after switching to fresh growth medium containing either 10 μg/ml insulin or 500 ng/ml IGF-I (Gropep, Adelaide, Australia) and a varying concentration of leptin, the cells were further induced to differentiate and maturate for 14 days, with the medium changed every other day.

Oil-Red-O staining

Lipid droplets contained in differentiating or mature adipocytes were stained by the Oil-Red-O method as described by Green and Kehinde [26]. In brief, cultured cells were washed twice with PBS, fixed for 30 minutes with 10% formalin and washed twice with distilled water prior to staining. Lipid droplets within the cell were stained for 30 min using 0.3% filtered Oil-Red-O solution in 60% isopropanol (Sigma Chemical Co.), after which the cells were washed twice with distilled water and photographed under the microscopic field.

Determination of lipolytic and lipid-accumulating activities

Concentrations of glycerol released into the culture

Table 1. Primer Sequences and PCR conditions

Item	Primer Sequences[a]	PCR conditions						Cycles	Size (bp)
		Denaturation		Annealing		Extension			
		Temp[b]	Time[c]	Temp	Time	Temp	Time		
IGF-I	F5'-GGA CCA GAG ACC CTT TGC GGG G-3' R5'-GGC TGC TTT TGT AGG CTT CAG TGG-3'	94	45	62	45	72	30	35	210
TGF-β	F5'-ACC GCA ACA ACG CCA TCT AT-3' R5'-GTA ACG CCA GGA ATT GTT GC-3'	94	20	55	20	72	30	35	200
C/EBP-α	F5'-TTA CAA CAG GCC AGG TTT CC-3' R5'-CTC TGG GAT GGA TCG ATT GT-3'	94	30	62	30	72	30	30	232
PPAR-δ	F5'-ATG GAA CAG CCA CAG GAG GAG-3' R5'-GAC ATT CCA TGT TGA GGC TGC-3'	94	30	53	30	72	30	30	220
PPAR-γ	F5'-GGT GAA ACT CTG GGA GAT TC-3' R5'-CAA CCA TTG GGT CAG CTC TC-3'	94	30	53	60	72	30	30	268
β-actin	F5'-GTG GGC CGC TCT AGG CAC CAA-3' R5'-CTC TTT GAT GTC ACG CAC GAT TTC-3'	94	30	60	30	72	120	25	540

[a] F = forward; R = reverse.
[b] Temperature (°C).
[c] Seconds.

media of the 3T3-L1 cells were determined as a measure of lipolysis using a GPO-Trinder colorimetric assay kit (Sigma Chemical Co.). Determination of the cellular content of triglycerides (TG), a measure of lipid accumulation, began with washing the cultured cells twice with PBS followed by scraping and homogenization in 25 mM Tris-HCl (pH 7.5)/1 mM EDTA using Ultrasonic Processor. The TG in the cell lysate was extracted with chloroform-methanol (2 : 1, v/v) and quantified using the GPO-Trinder Triglyceride kit (Sigma Chemical Co.). Alternatively, the cellular TG content was semi-quantitated by staining the cultured cells with Oil-Red-O, followed by dissolution of the stained dye in 1-ml isopropanol for 15 min on a shaker and spectophotometry at 490 nm [27, 28].

Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from cultured cells following the modified guanidine thiocyanate method [29]. First-strand complementary DNA (cDNA) was synthesized using 1 μg of total RNA as template, 0.3 μg of a mixture of random primers (Gibco) and AccPower™ RT Premix (Bioneer Co., Daejeon, Korea) in a total volume of 20 μl according to the manufacturer's instruction as previously described [30]. The targeted fragment of cDNA per each of the differentiation-associated genes (Table 1) was amplified by PCR using 5 μl of the RT product, 20 pmoles of each of the primer pair and the AccuPower™ PCR premix (Bioneer) under the conditions also shown in Table 1. The PCR products were separated by electrophoresis on 1.5 to 2% agarose gels and visualized by staining with ethidium bromide. The stained PCR band was scanned using a 1-D image analyzer (Kodak Co., Rochester, NY, USA) and the resulting densitometric numerical was normalized to that of β-actin.

Statistical analysis

All the quantitative data were analyzed by the General Linear Model Procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The statistical model included the dose of leptin or its equivalent (none, IGF-I and IGF-I plus leptin treatments in the insulin-free culture), day of incubation and an interaction of the two fixed errors.

Results

Effect of leptin on proliferation of preadipocytes

The effect of leptin on proliferation of 3T3-L1

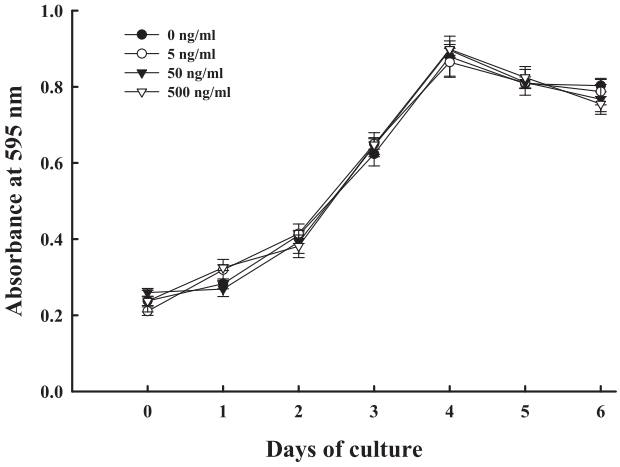

Fig. 1. Effects of leptin on 3T3-L1 preadipocyte proliferation. The 3T3-L1 cells were pre-cultured in 0.2 ml of a growth medium containing 10% calf serum at a density of 1.0×10^4 cells/well in 96-well plates and switched to the growth medium containing indicated concentration of leptin. The mitotic activity of the cultured cells was measured by the MTT assay in which following a 2-hr incubation of the cells with MTT at 37°C and removal of the MTT-treated medium, each well received 0.15-ml dimethyl sulfoxide and spectrophotometry at 595 nm on an enzyme-linked immunosorbent assay plate reader. Data are means ± SE of four wells. Effect of the day of culture was significant ($p<0.01$).

preadipocytes was measured by the MTT assay during a 6-day culture following a 1-day pre-culture. The number of the cells increased linearly ($p<0.01$) during the first and second two days with different slopes and decreased slightly after day 4 (Fig. 1). Addition of leptin to the culture medium to 5, 50 or 500 ng/ml had no effect on the measure of cell proliferation.

Induction of differentiation of preadipocytes into adipocytes

The 3T3-L1 preadipocytes were induced to differentiate into adipocytes in a growth medium containing MDI (methylisobutylxanthine, dexamethasone and insulin) for two days and further induced to differentiate and maturate in the growth medium supplemented only with insulin for 14 days. Following the hormonal induction of differentiation, the 3T3-L1 cells, which had contained no discernible vesicles prior to differentiation (Fig. 2A), exhibited a well-developed, apparently lipid-filled vesicular structure typical of the morphology of mature cells (Fig. 2B). Consistent with this dramatic morphological change, the differentiated

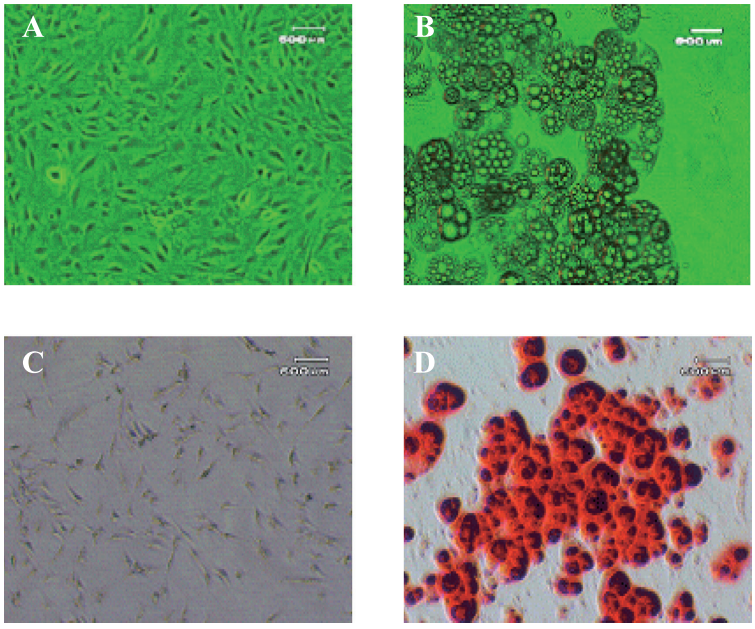

Fig. 2. Differentiation and maturation of 3T3-L1 preadipocytes. *Panels A and B*, Morphology of 3T3-L1 cells (×40) before (A) and after differentiation and maturation (B). The 3T3-L1 preadipocyte, which had been cultured to confluence in a growth medium containing 10% calf serum (A), was induced to initiate differentiation for two days in the growth medium supplemented with MDI (0.5 mM methylisobutylxanthine, 1 μM dexamethasone and 10 μg/ml insulin) followed by further induction of differentiation and maturation in the growth medium supplemented with 10 μg/ml insulin for 14 days (B), with the medium changed every other day. *Panels C and D*, Oil-Red-O staining. Lipid droplets in the undifferentiated preadipocytes (A) and differentiated mature adipocytes (B) were stained with Oil-Red-O. Note the intracellular lipid droplets in the mature adipocytes (B & D), but not in the preadipocytes (A & C).

mature cells (Fig. 2D), but not undifferentiated cells (Fig. 2C), reacted with the Oil-Red-O dye to yield compact red stains, which indicates that the vesicles in the former were indeed filled with lipid droplets.

Effects of leptin on lipolysis and TG accumulation during adipocyte differentiation and maturation

Following hormonal induction of differentiation of the preadipocytes, the lipid-accumulating and lipolytic activities of the differentiating and maturing cells were determined by measuring the TG content in the cellular homogenate and the concentration of glycerol released into the medium, respectively. Furthermore, the TG content was also monitored by staining the intracellular lipid vesicles with Oil-Red-O.

The 3T3-L1 cells exhibited increasing numbers of Oil-Red-O-stainable lipid vesicles with increasing days in culture (Fig. 3, *top panel*), indicating that the cells differentiated and maturated during the culture. Glycerol concentration in the medium also increased linearly with increasing days of culture (Fig. 3, *middle panel*; $p<0.01$ of day effect). Moreover, leptin added to the medium caused an increase in glycerol concentration in a dose-dependent manner ($p<0.01$ of leptin dose effect). The TG content in the homogenate of the

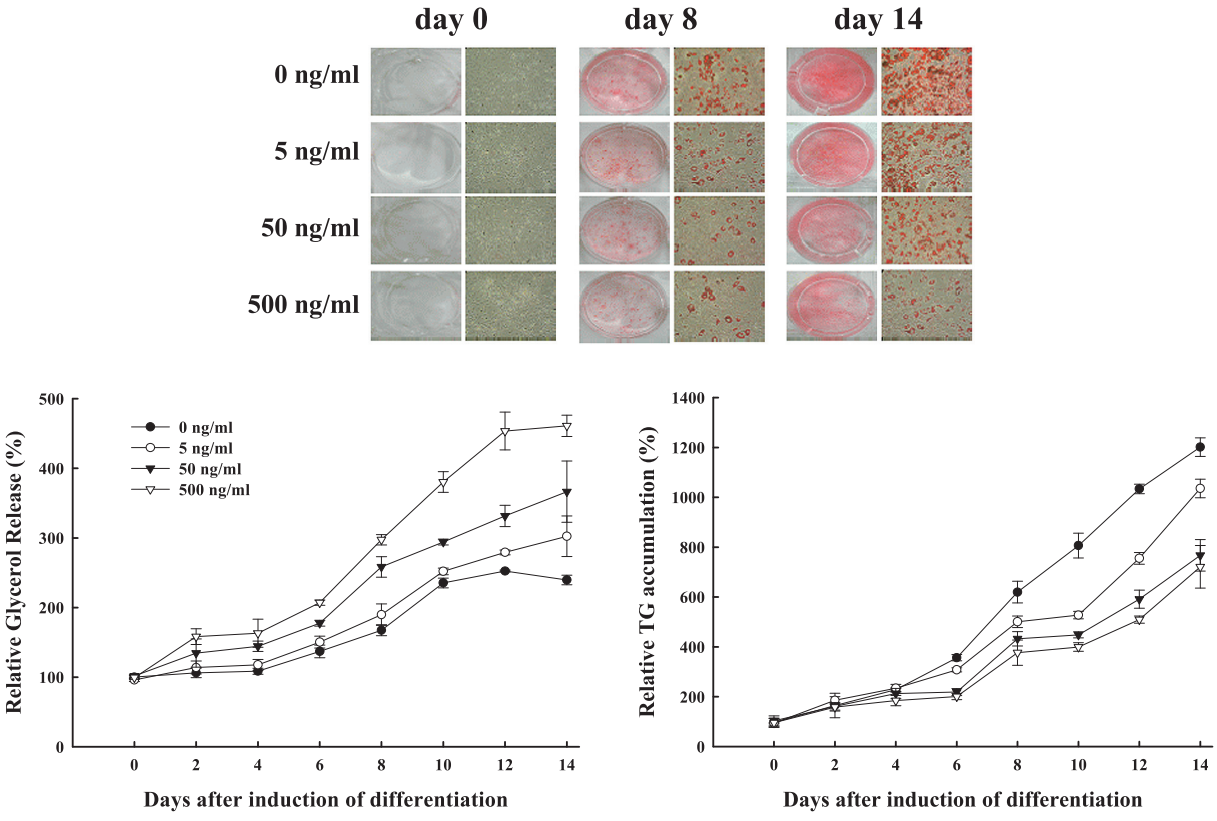

Fig. 3. Effects of leptin on lipid accumulation and lipolysis during 3T3-L1 cell differentiation and maturation. *Upper panel*, Morphological change and lipid accumulation during adipocyte differentiation and maturation. The 3T3-L1 preadipocytes were initiated to differentiate in a growth medium (DMEM/10% calf serum) supplemented with MDI (0.5 mM methylisobutylxanthine, 1 µM dexamethasone and 10 µg/ml insulin) for two days, after which (day 0) the cells were further induced to differentiate and maturate for 14 days in the growth medium supplemented with 10 µg/ml insulin and the indicated concentration of recombinant mouse leptin, with the medium changed every other day. Morphology and accumulation of lipid droplets of the cells were monitored by microscopic examination before (left columns) and after (right columns) Oil-Red-O staining, respectively, on days 0, 8 and 14. *Middle and bottom panels*, Lipolysis and triglyceride (TG) accumulation during adipocyte differentiation and maturation. Lipolysis (*middle*) and TG accumulation (*bottom*) of the cells at the indicated days were monitored by measuring the glycerol concentration of the conditioned culture medium and the TG content of the cellular homogenate, respectively, using commercially available assay kits. Data, which are expressed as percentages relative to that at day 0 in the absence of added leptin (100%), are means ± SE of three replicates. Effects of leptin dose and day after induction of differentiation were significant ($p<0.01$) in glycerol release; in TG accumulation, effects of these two fixed errors as well as an interaction of them were significant ($p<0.01$).

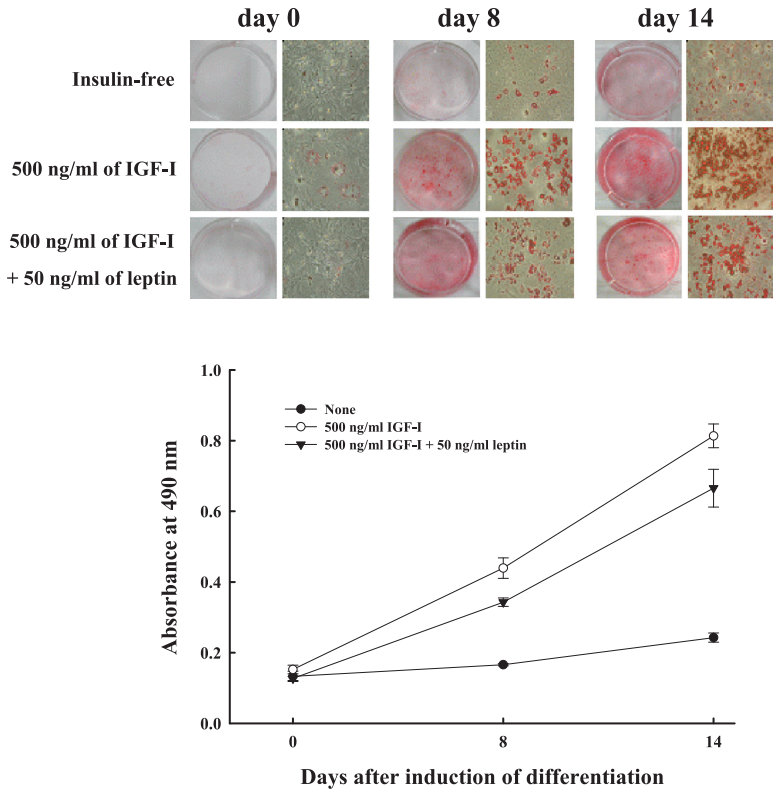

Fig. 4. Effects of leptin on IGF-I-stimulated lipid accumulation during 3T3-L1 cell differentiation and maturation. *Upper panel*, Microscopic examination. Following a 2-day induction of differentiation in a growth medium (DMEM/10% calf serum) supplemented with DMI (0.5 mM methylisobutylxanthine, 1 μM dexamethasone and 10 μg/ml insulin), the 3T3-L1 cells were further induced to differentiate and maturate in the growth medium in the absence or presence of 500 ng/ml IGF-I ± 50 ng/ml leptin for 14 days, with the medium changed every other day. Morphology and accumulation of lipid droplets of the cells were monitored by microscopic examination before (left columns) and after (right columns) Oil-Red-O staining, respectively, on days 0, 8 and 14. *Lower panel*, Determination of the content of cellular triglycerides. The amount of triglycerides accumulated in the cell at the indicated day was semi-quantitatively monitored by Oil-Red-O staining followed by dissolution of the stained dye in isopropanol and spectophotometry at 490 nm. Data are means ± SE of three replicates. Effects of the treatment and day were significant (p<0.01).

cells also increased during adipocyte differentiation and maturation (Fig. 3, *lower panel*). The cellular TG content decreased dose-dependently in response to leptin added to the medium (p<0.01 of dose effect).

Leptin inhibited IGF-I-stimulated lipid accumulation of the cell as well. As shown in Fig. 4, the cellular TG content barely changed during the 14 days in the insulin-free culture in the absence of IGF-I, whereas in the culture in the presence of 500 ng/ml IGF-I, the TG content increased (p<0.01) several folds with increasing days in culture. The IGF-I-stimulated increase of cellular TG content diminished following addition of leptin to the culture medium at a dose of 50 ng/ml which was effective in inhibiting the TG accumulation of the cells cultured in the insulin-containing medium in the previous experiment.

Gene expression of differentiation-associated growth factors and transcription factors

Gene expression of a number of growth factors and transcription factors during the adipocyte differentiation and maturation was monitored by RT-PCR (Fig. 5). The IGF-I gene expression increased up to day 6 and declined (p<0.01 of day effect). Addition of leptin to the medium at 50 ng/ml caused a decreased expression of this gene (p<0.01). The expression of TGF-β1 gene increased over 10 folds up to day 8 and tended to plateau during the later phase of the 14-day culture (p<0.01 of day effect). The C/EBP-α gene expression, which was almost undetectable at days –2 and 0, was induced dramatically up to day 6 to 8 (p<0.01 of day effect), whereas PPAR-δ gene expression increased transiently during the early phase of the culture

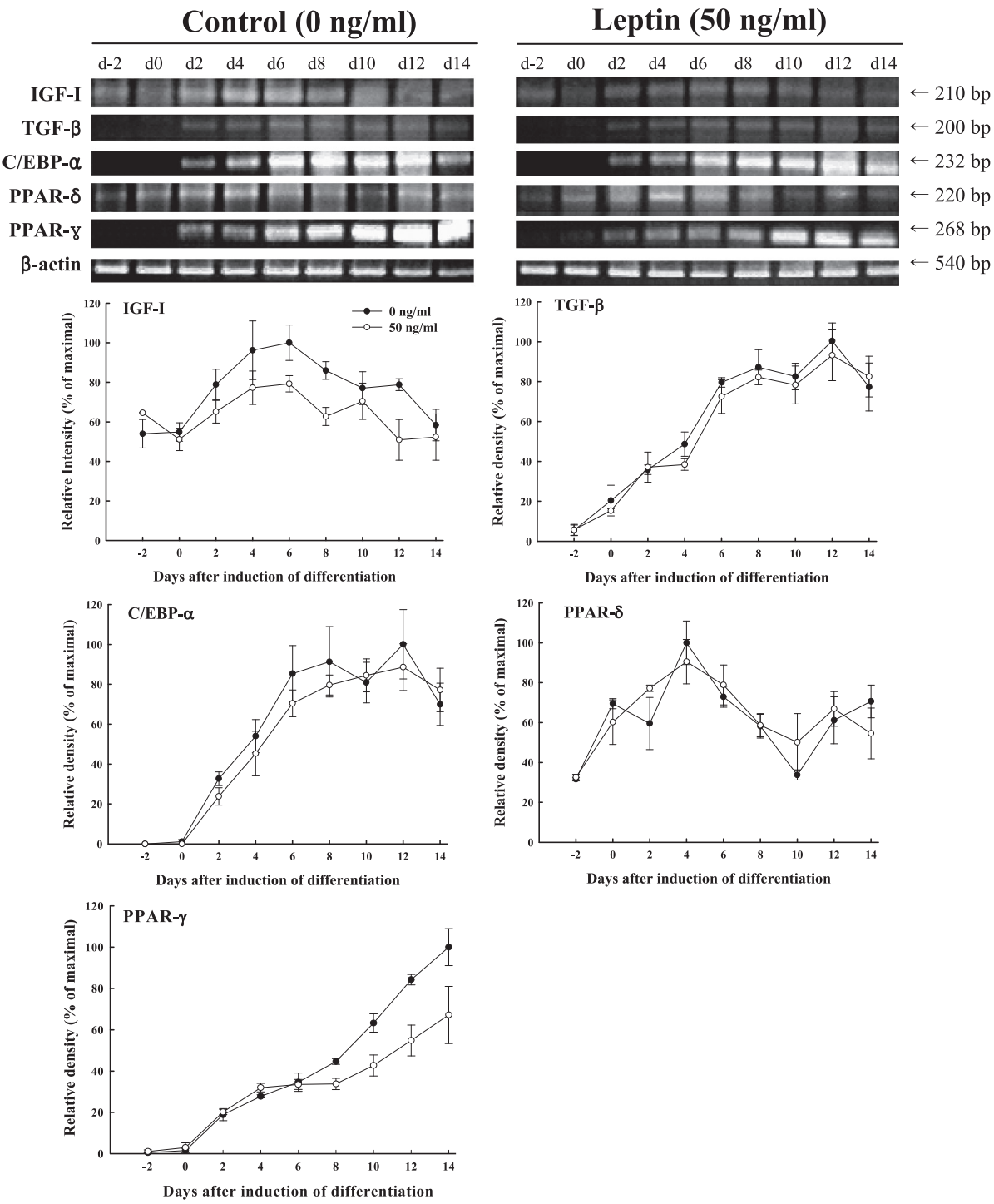

Fig. 5. Gene expression of differention-associated growth factors and transcription factors during 3T3-L1 cell differentiation and maturation. *Upper*, Reverse transcription-polymerase chain reaction. The 3T3-L1 preadipocytes were induced to differentiate in a growth medium (DMEM/10% calf serum) supplemented with MDI (0.5 mM methylisobutylxanthine, 1 μM dexamethasone and 10 μg/ml insulin) for two days between d-2 and d0 in the figure. The cells were allowed to further differentiate and maturate up to the indicated day in the growth medium supplemented with 10 μg/ml insulin in the absence or presence of 50 ng/ml leptin, with the medium changed every other day. The RT-PCR for the indicated growth factor or transcription factor was performed under the conditions shown in Table 1, followed by electrophoresis of the PCR products on agarose gels and staining with ethidium bromide. *Lower*, Temporal change of gene expression during differentiation and maturation. The ethidium bromide-stained PCR products in the *upper* panel were scanned and the resulting densitometric numericals were normalized to that of β-actin of the corresponding RNA sample. Data are means ± SE of three replicates. The effect of day was significant in TGF-β, C/EBP-α and PPAR-δ ($p<0.01$). Both effects of the day and leptin were significant ($p<0.01$) in IGF-I; in PPAR-γ, both effects as well as an interaction of them were significant ($p<0.01$).

($p<0.01$ of day effect). Leptin added to the medium had no effect on gene expression of these transcription factors or TGF-β1. The PPAR-γ gene expression, which, like that of C/EBP-α, was almost undetectable at the beginning of the differentiation, was induced remarkably during the entire period. Expression of this gene increased with increasing days in culture ($p<0.01$ of day effect). Moreover, leptin caused a significant decrease ($p<0.01$) of the gene expression, which was evident from day 10 of the culture.

Effects of leptin on lipid metabolism in mature adipocytes

Effects of leptin on glycerol release and TG accumulation of mature 3T3-L1 adipocytes during 24- and 48-hr cultures were next examined following a total of 16-day differentiation and maturation of the cells. Consistent with previous results with differentiating/maturing adipocytes, leptin added to the culture medium enhanced glycerol release into the medium ($p<0.01$ of dose effect) in mature adipocytes (Fig. 6, *upper*). Moreover, this effect was evident at 50 and 500 ng/ml concentrations ($p<0.05$ and $p<0.01$ in 24- and 48-hr cultures, respectively), but not at 5 ng/ml. The concentration of glycerol released into the medium increased slightly following the 48-hr culture vs. 24-hr culture ($p<0.05$).

Leptin added to the medium inhibited TG accumulation ($p<0.01$ of dose effect) in mature adipoytes as well (Fig. 6, *lower*). This effect of leptin was also dose-dependent within a few fold range, although relative effects of the graded doses of this peptide were slightly different between the 24- and 48-hr cultures. However, the cellular TG content did not increase following the 48-hr culture vs. 24-hr culture.

Discussion

The development and metabolism of the adipocyte lineage are regulated by a variety of transcription factors and hormonal agents including leptin and growth factors [31, 32]. In the present study, effects of leptin on lipid metabolism and gene expression related to cellular development during differentiation and maturation of 3T3-L1 preadipocytes were investigated.

The lack of effect of leptin at a dose of 5 to 500 ng/ml (0.3 to 30 nM) on mitosis of 3T3-L1 cells during the first two days was similar to the results of Ambati *et al.* [33], although in the latter study, this regulatory peptide exhibited a slightly anti-mitotic effect within some 10% range irrespective of its dose between 1 and 1,000 nM. These results, however, are different from those of Zwirska-Korczala [34] in which proliferation of the cells in the presence of 0.01 to 1,000 nM leptin decreased 36% compared with that of control during a 24-h period. Conflicting results regarding the effect of leptin on mitosis have also been reported in primary preadipocytes. In this cell type, leptin has been reported to stimulate cell proliferation at supra-physiological concentrations over 100 ng/ml [35, 36], to have a biphasic mitogenic effect at 50 to 500 ng/ml of supra-physiological concentrations [37], but to have no effect on mitosis at physiological (5 to 10 ng/ml) concentrations [36, 37]. Obviously, more studies are necessary to clarify the effect of leptin on proliferation of preadipocytes including the 3T3-L1 cell line.

The 3T3-L1 preadipocytes underwent the terminal differentiation during the early phase of the 16-day hormonal induction of differentiation and subsequent maturation, which was indicated by the remarkable induction of the terminal differentiation makers PPAR-γ and C/EBP-α during the first four to six days of the culture. Moreover, the differentiated adipocytes developed to fully mature cells during the mid to late phase of the culture as indicated by the remarkable increase of the lipid vesiculature during this period. In these processes, leptin inhibited insulin- or IGF-I-stimulated cellular lipid accumulation and enhanced glycerol excretion of the 3T3-L1 cell. This is consistent with the anti-lipogenic action of this peptide in differentiating 3T3-L1 preadipocytes [33] as well as with its anti-lipogenic and lipolytic effects in differentiated 30A5 adipocytes [38] and primary rodent adipocytes [39, 40], suggesting that leptin is likely an autocrine/paracrine regulator of lipid metabolism in differentiating as well as mature adipocytes. However, these results are somewhat different from those of a study with human primary adipocytes in which leptin had no effect on lipolysis in spite of its inhibitory effect on insulin-stimulated lipogenesis [41]. More studies are therefore necessary to unequivocally determine a possible species- or cell-type specificity of the lipolytic responsiveness of the adipocyte to leptin.

The hormonally induced expression of PPAR-γ and C/EBP-α genes concomitant with the induction of differentiation is consistent with the fact that these are

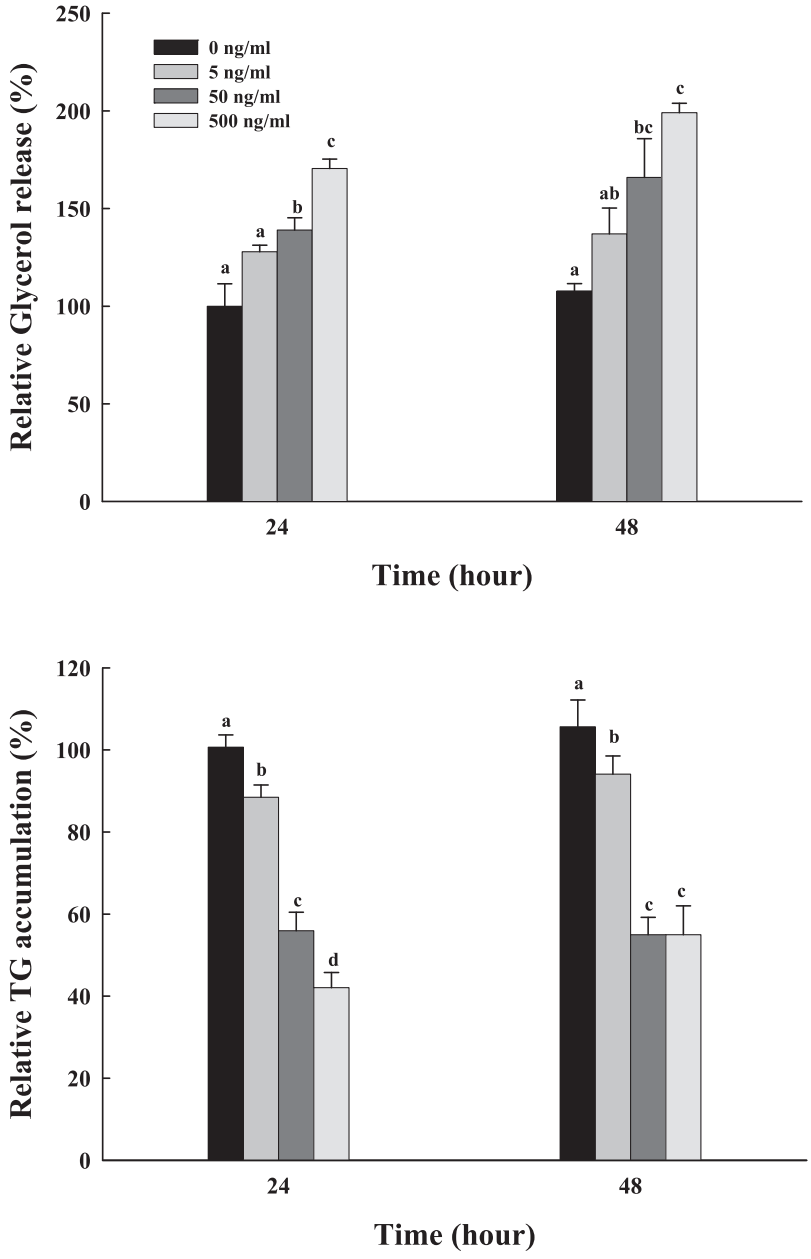

Fig. 6. Effects of leptin on glycerol release and triglyceride accumulation in mature 3T3-L1 adipocytes. Mature adipocytes following a total of 16-day differentiation and maturation of the 3T3-L1 preadipocytes were incubated for 24 or 48 hr in a growth medium (DMEM/10% calf serum) supplemented with 10 μg/ml insulin and an indicated concentration of leptin. Concentrations of glycerol released into the culture media and the triglyceride (TG) contents in the cellular homogenates were measured using commercially available kits. Data, which are means ± SE of three replicates, were expressed as percentages relative to the mean for the 24-hr incubation in the absence of added leptin (100%) in each variable. Effects of the dose of leptin and incubation time were significant ($p<0.01$ and $p<0.05$, respectively) in glycerol release, whereas in TG accumulation, only the effect of the dose of leptin was significant ($p<0.01$). Bars with different letters within each incubation time are different ($p<0.05$).

master transcription factors triggering the expression of adipocyte-specific genes responsible for the accumulation of triglycerides [11, 13]. Moreover, the transient increase of expression of PPAR-δ and IGF-I genes during early differentiation was consistent with previously reported results in this cell line [14, 42]. It also was noteworthy that the time-course of the expression of the known differentiation inhibitor TBF-

β was very similar to that of C/EBP-α, although biological significance of this remains to be studied.

Exogenous leptin inhibited the expression of PPAR-γ and IGF-I genes during the maturation and entire differentiation/maturation of the 3T3-L1 cell, respectively. This suggests that the anti-lipogenic and lipolytic effects of leptin may have been partly mediated by suppressing the expression of PPAR-γ and IGF-I genes, because PPAR-γ and IGF-I are a known stimulator of lipogenesis and an established lipogenesis stimulator and lipolysis inhibitor, respectively [5, 6]. Collectively, results of the present study also suggest that adipocyte differentiation and maturation may involve intricate interactions between IGF-I, leptin and PPAR-γ. Obviously, more studies are necessary to determine whether there exists this speculative autocrine/paracrine regulation of adipocyte development. Also what needs to be studied is the time-course of expression of the leptin receptor, because all the effects of leptin detected in the present and previous studies in 3T3-L1 cells [33, 34] are assumed to have been mediated through its cognate receptors whose expression has not been confirmed in any of these studies. In primary rat preadipocytes, two types of leptin receptors have been reported to be expressed to mediate the mitotic and metabolic actions of their ligand beginning from the pre-differentiation stage of the cell [35], suggesting that leptin receptors are likely to be expressed during the 3T3-L1 preadipocyte development as well.

Acknowledgment

This work was supported by grants of Korea Science and Engineering Foundation (MOSEF)/ Ministry of Science and Technology (MOST) (No. M10641000013–06N410–01310) and the Ministry of Knowledge Economy/Korea Institute of Industrial Technology Evaluation and Planning (ITEP) through the Regional Animal Industry Center at Jinju National University, Jinju, Korea.

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