BIOCHEMICAL PHARMACOLOGY 73 (2007) 378–384

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Effects of leptin on apoptosis and adipogenesis in 3T3-L1 adipocytes

Suresh Ambati [a], *Hye-Kyeong Kim* [a,1], *Jeong-Yeh Yang* [a], *Ji Lin* [a,2], *Mary Anne Della-Fera* [a], *Clifton A. Baile* [a,b,*]

[a] *Department of Animal & Dairy Science, University of Georgia, Athens, GA 30602-2771, USA*
[b] *Department of Food and Nutrition, University of Georgia, Athens, GA 30602-2771, USA*

ARTICLE INFO

Article history:
Received 25 August 2006
Accepted 9 October 2006

Keywords:
Leptin
Adipocyte
Apoptosis
Adipogenesis
Viability

ABSTRACT

Leptin has been demonstrated to induce adipose tissue apoptosis, which can contribute to the decrease of adiposity, after either central nervous system or peripheral administration. However, it is not known whether leptin acts only centrally to initiate a signal or can also act directly on adipocytes to induce apoptosis. The objective of this study was to determine the direct effect of leptin on adipocyte apoptosis and adipogenesis in vitro using 3T3-L1 cell lines. An ELISA for single stranded DNA, which is highly specific for apoptotic cells, was used to quantify apoptosis. Preconfluent preadipocytes treated with 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} M leptin showed inhibitory effects on cell viability, and similar observations were also found in maturing preadipocytes treated during day 0–2 and day 2–4 of maturation. After 48 h incubation with 10^{-6} M leptin, LDH release was increased by 24.3% ($p < 0.05$) in preconfluent preadipocytes and by 108.5% ($p < 0.01$) in maturing preadipocytes. However, ssDNA analysis revealed no increased apoptosis in preconfluent or maturing preadipocytes or in mature adipocytes treated with leptin. Leptin significantly reduced lipid accumulation and GPDH activity in maturing preadipocytes, demonstrating an inhibitory effect of leptin on adipogenesis. These results indicate that leptin does not act directly to induce adipocyte apoptosis, but can act directly to inhibit maturation of preadipocytes.

1. Introduction

With the tremendous increase in the prevalence of obesity and diabetes, understanding the critical aspects in adipose tissue growth and physiology is becoming increasingly important. Leptin, a hormone produced by adipocytes, plays a vital role in the regulation of body energy [1–3]. Although suppression of food intake is an important centrally mediated effect of leptin, it also functions both peripherally and centrally to orchestrate complex metabolic changes in a number of organs and tissues, altering nutrient flux to favor lipid mobilization over lipid storage [1]. The extreme depletion of adipose depots and prolonged recovery time for repletion of fat stores following long-term leptin treatment demonstrate the profound effect of leptin on adipose tissue.

In general, adipose tissue mass is determined by a balance of lipolysis, lipogenesis, and adipocyte proliferation. Recent evidence demonstrates that leptin can decrease adiposity by

* *Corresponding author at*: 444 Edgar L. Rhodes Center for Animal and Dairy Science, University of Georgia, Athens, GA 30602-2771, USA. Tel.: +1 706 542 2771; fax: +1 706 542 7925.
E-mail address: cbaile@uga.edu (C.A. Baile).
[1] Current address: Department of Food and Nutrition, Seoul National University, Seoul, Republic of Korea.
[2] Current address: Oncologic, Inc., Berkeley, CA 94710.
0006-2952/$ – see front matter
doi:10.1016/j.bcp.2006.10.009

triggering an apoptotic process in addition to activating lipolysis [4–6]. Adipose tissue from rats given cerebral ventricular injections of leptin demonstrated features of apoptosis, including internucleosomal fragmentation of genomic DNA, elevated levels of DNA strand breaks, reduction in total DNA content, and cellular volume [7]. Although apoptosis has been extensively studied in other cell types, apoptosis of adipose tissue has been described only relatively recently. In some forms of malignancy, rapid weight loss is associated with loss of adipocytes by apoptosis [8]. There also appears to be a depot-specific susceptibility to apoptosis, with visceral adipose tissue being more susceptible to apoptotic stimuli than subcutaneous adipose tissue [9].

The mechanisms of leptin-induced adipose tissue apoptosis are not fully understood. It has been suggested that leptin-induced adipose tissue apoptosis is mediated centrally by stimulation of sympathetic nervous (SNS) activity; based on the fact that leptin increases SNS output and centrally-effective doses of leptin are much lower than peripherally effective doses [10]. In addition, it has been shown that adipose tissue contains both alpha and beta adrenergic receptors, and we showed previously that the beta-2 adrenergic agonist, clenbuterol, increased adipose tissue apoptosis in vivo [11]. Nevertheless, it is difficult to assume leptin-induced adipose apoptosis to be mediated only centrally, since we cannot exclude the possibility that leptin can act directly on adipocytes. However, the direct effect of leptin on adipocyte apoptosis in vitro has not been previously investigated.

In this experiment we used 3T3-L1 adipocytes and preadipocytes to determine the direct effects of leptin in vitro. The objective of our study was to determine whether leptin can act directly on adipocytes to induce apoptosis and inhibit adipogenesis, a marker of differentiation.

2. Materials and methods

2.1. Chemicals

Recombinant mouse leptin was a gift of Professor Arieh Gertler (Protein Laboratories Rehovot (PLR) Ltd., Rehovot, Israel); insulin, dexamethasone, and 3-isobutylxanthine were obtained from Sigma (St. Louis, MO). The tissue culture materials were obtained from GIBCO (Grand Island, NY). Leptin was reconstituted using 10^{-4} M leptin stock solution prepared in PBS, filter sterilized, and stored at −20 °C. Cells were treated with leptin at the concentration and times indicated in each assay.

2.2. Cell culture

3T3-L1 mouse embryo fibroblasts (American Type Culture Collection, Manassas, VA) were thawed and cultured as per the protocol described by Hemati et al. [12]. Briefly, cells were cultured in Dulbecco's modified Eagles's Medium (DMEM) containing 10% bovine calf serum (BCS), 1% penicillin–streptomycin (10,000 U/ml) and 1% (v/v) 100 mM pyruvate at 37 °C in 95% air–5% CO_2. Two days after cells became confluent (day 0), they were stimulated to differentiate with 0.5 μM isobutylmethylxanthine (IBMX), 1 μM dexamethasone, and 167 nM insulin (MDI) added to DMEM media containing 10% fetal bovine serum (FBS) for 2 days (day 0–2). Cells were then maintained in 10% FBS/DMEM medium with 167 nM insulin for another 2 days (day 2–4), followed by culturing with 10% FBS/DMEM medium for an additional 4 days (day 4–8), at which time more than 90% of cells were mature adipocytes with accumulated fat droplets. For the following experiments treatment of preconfluent preadipocytes began one day after they were seeded in 96-well plates at a density of 2500 cells/well. Maturing preadipocytes were treated for 48 h periods during the differentiation phase (day 0–2, day 2–4, day 4–6). Treatment of mature adipocytes began on day 9.

2.3. Assessment of cell viability

The CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI) was used to assess the number of viable cells. This assay is based on the mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to formazan, which is directly proportional to the number of living cells in culture. Preconfluent 3T3-L1 preadipocytes were seeded in 96-well plates at a density of 2500 cells/well. Control and leptin (10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} M) treatments were subsequently added 24 h after the cells were seeded and viability was measured after 6, 12, 24 and 48 h. Mature adipocytes were treated on day 9 for 6, 12, 24 and 48 h. Maturing preadipocytes were treated for 48 h during day 0–2, 2–4 and 4–6 of differentiation.

2.4. Lactate dehydrogenase (LDH) activity as a measure of cytotoxicity

The presence of LDH in the spent media relative to its activity in adherent cells has been used as an index of direct cytotoxicity of exogenous material. Preadipocytes were seeded at a density of 2500 cells/well and leptin (10^{-8} and 10^{-6} M) was added 24 h after cell seeding. In maturing preadipocytes, leptin (10^{-8} and 10^{-6} M) treatments were added on day 0 of differentiation. In both cases, after 48 h culture, LDH assay was performed using CytoTox-ONE™ assay (Promega, Madison, WI) following the manufacturer's instructions. LDH activity released in control and leptin-treated cells was expressed as a percent of the maximum LDH released in lysed nontreated cells (% cytotoxicity).

2.5. Apoptosis assay

For the assessment of apoptosis, we used the ApoStrand™ ELISA Apoptosis Detection Kit (Biomol, Plymouth Meeting, PA). The ApoStrand™ ELISA Apoptosis Detection Kit detects single stranded DNA, which occurs in apoptotic cells but not in necrotic cells or in cells with DNA breaks in the absence of apoptosis [13,14].

Tests were performed in cells grown in 96-well plates. For mature adipocytes, cells were seeded (5000 cells/well), grown to maturity and leptin treatments (10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} M) were added on day 8 of differentiation. For preadipocytes, a seeding density of 2500 cells/well was used, and cells were cultured overnight before treatment. Cells were incubated with leptin (10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} M) for 24 and 48 h. For maturing preadipocytes, a seeding density of 2500 cells/well was used and

cells were grown until confluent before treatment. Leptin was added with the induction medium to final concentrations of 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} M and cells were incubated for 48 h during days 0–2, 2–4 and 4–6. Controls without added leptin were included in all assays. Cells were fixed for 30 min and assayed according to the manufacturer's instructions.

2.6. Quantification of lipid content by AdipoRed™ assay

Lipid content was measured using a commercially available kit according to the manufacturer's instructions (AdipoRed™ Assay Reagent, Cambrex BioScience Walkersville, Inc.). AdipoRed reagent is a solution of the hydrophilic stain Nile Red. In brief, for the quantification of adipogenesis, cells were incubated with control or leptin (10^{-9} 10^{-8} 10^{-7} and 10^{-6} M) for 48 h periods (day 0–2, day 2–4 or day 4–6) during the adipogenic phase, and intracellular lipid content was measured by AdipoRed assay on day 6.

2.7. Oil-Red-O staining

Cells were treated with control or leptin (10^{-8} and 10^{-6} M) from days 0 to 6 of adipogenesis. Medium was changed every 2 days. On day 6, cells were stained with Oil-Red-O as described elsewhere [15]. In brief, dishes were washed with cold PBS and fixed in 10% neutral formalin. After two changes of propylene glycol, Oil-Red-O was added with agitation for 7 min, followed by washing in 85% propylene glycol. The dishes were then rinsed in distilled water and counterstained with hematoxylin. For each dish, three images were taken and analyzed for lipid droplet size, percentage lipid area, and total droplet number with ImagePro Plus 5.0 software (MediaCybernetics, Silver Spring, MD).

2.8. Glycerol-3-phosphate dehydrogenase (GPDH) assay

GPDH assay was used as a marker of late adipocyte differentiation. After 6 days of culture with leptin (10^{-8} and 10^{-6} M) or control in the differentiation medium (day 0–6), the media were discarded and cells were rinsed thrice with PBS, scraped into 0.5 ml ice-cold sucrose buffer containing 0.28 M sucrose, 5 mM Tris. 1 mM EDTA, 0.002% β-mercaptoethanol, and stored at −70 °C until assayed. The cells were sonicated with 3 blasts for 15 s and centrifuged at 10,000 × *g* for 10 min at 4 °C, and the resulting supernatants were used for GPDH assays according to Wise and Green [16]. The cytosolic protein concentration was determined using the BCA Protein Assay (Pierce Biotechnology, Inc., Rockford, IL). Activities were measured as mUnits GPDH per mg of protein (1 mU being equal to the oxidation of 1 nmol of $NADH_2$/min) and data are shown as % control.

2.9. Statistical analysis

One-way or two-way analysis of variance (ANOVA) was used to determine significance of treatment, time and treatment × time effects. Differences among treatment means were determined by Fisher's least significant difference test, and p values <0.05 are considered significant. All values are expressed as means ± S.E.M. for data analysis.

3. Results

3.1. Effect of leptin on number of viable cells

In preconfluent preadipocytes there were significant treatment, time and treatment × time effects ($p < 0.01$ for each). Fig. 1 shows significant differences among treatments within the specific time periods. All leptin treatments in preconfluent preadipocytes resulted in significant inhibition of cell viability after 12, 24 and 48 h, as determined by the MTS assay (Fig. 1A). Leptin had no effect on viability at any time in mature adipocytes (data not shown). In maturing preadipocytes, there were significant treatment and treatment x time effects ($p < 0.01$ for each); although there was not a significant overall treatment effect, Fisher's LSD test showed that there were some significant differences among means for the day 0–2 and day 2–4 treatment periods (Fig. 1B).

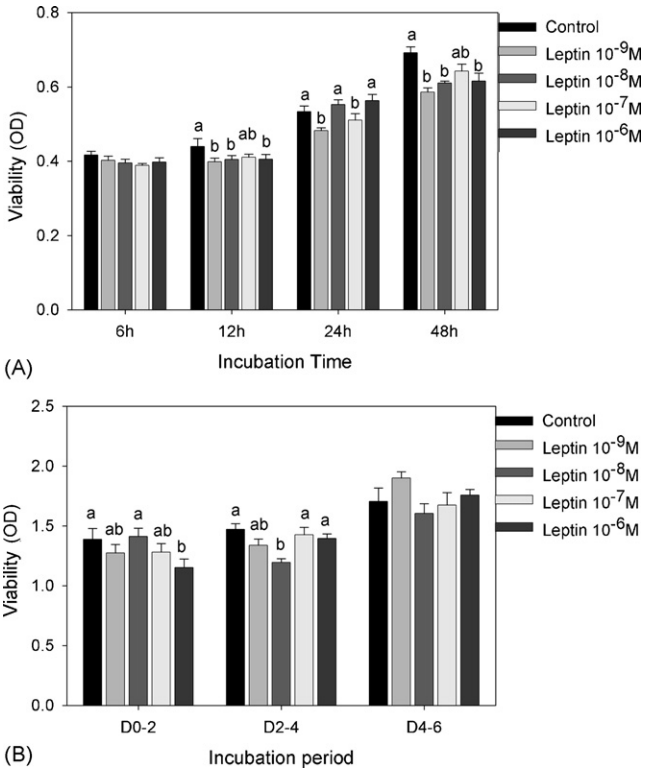

Fig. 1 – Effect of leptin on viability of preconfluent preadipocytes and maturing preadipocytes. (A) 3T3-L1 preconfluent preadipocytes were incubated with leptin (10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} M) for 6, 12, 24 and 48 h. (B) Maturing preadipocytes were incubated with leptin (10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} M) for 48 h periods during on days 0–2, 2–4, or 4–6 of differentiation. Cell viability after leptin treatment was determined by the MTS colorimetric assay. Assays were performed in eight replicates for each treatment and experiments were repeated two to three times. Values are means ± S.E.M. Within a time period, means with different letters (a and b) are different, $p < 0.05$.

Table 1 – LDH release in preconfluent and maturing preadipocytes after incubation with leptin for 48 h (% cytotoxicity, mean ± S.E.M.)

	Control	Leptin ($\times 10^{-8}$ M)	Leptin ($\times 10^{-6}$ M)
Preconfluent	7.6 ± 0.3 a	7.0 ± 0.3 a	9.4 ± 0.3 b
Maturing	7.2 ± 0.4 ax	12.2 ± 0.7 by	15.1 ± 0.8 cy

Means with different letters are different: $^{a,b}p < 0.05$; $^{x,y,z}p < 0.01$.

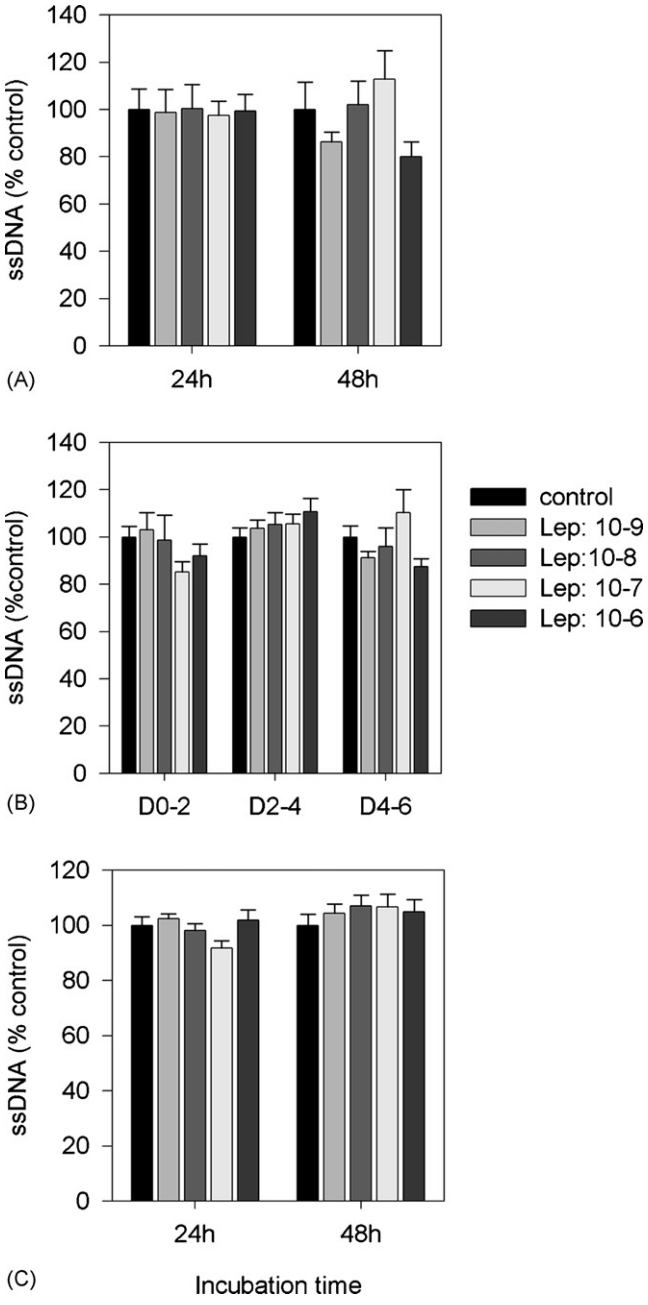

Fig. 2 – Effect of leptin on apoptosis (% control) in preconfluent preadipocytes, maturing preadipocytes, and lipid filled adipocytes. Cells were treated with leptin (10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} M) for the times indicated. Apoptosis was quantified by ssDNA ELISA. Assays were performed in eight replicates for each treatment and experiments were repeated two to three times. Values are means ± S.E.M.

3.2. *Effect of leptin on LDH activity*

LDH activity was measured to determine whether the decrease in viability caused by leptin was due to a cytotoxic effect on preadipocytes. LDH release into the culture medium was significantly increased after 48 h incubation with 10^{-6} M leptin in preconfluent preadipocytes and with both 10^{-8} and 10^{-6} M in maturing preadipocytes (Table 1).

3.3. *Effect of leptin on apoptosis*

Leptin had no effect on apoptosis of either preconfluent preadipocytes or mature adipocytes after 24 or 48 h incubation (Fig. 2).

3.4. *Effect of leptin on maturation and lipid accumulation*

Leptin significantly inhibited lipid accumulation in maturing preadipocytes when cells were incubated during days 0–2, 2–4 and 4–6, although it was more effective during days 0–2 and 2–4 (Fig. 3). Similar results were observed using Oil-Red-O to visualize lipid after cells were incubated with leptin treatments for 6 days (days 0–6). The representative images demonstrated that leptin suppressed lipid accumulation (Fig. 4A). This was also supported by quantitative data on image analysis, where leptin dose-dependently decreased the lipid percent area and the number of lipid droplets in maturing preadipocytes (Fig. 4B). Leptin also reduced GPDH activity ($p < 0.05$) in maturing preadipocytes in a dose dependent manner (Fig. 5).

4. Discussion

Although leptin is produced by adipose tissue, it plays a mediator role in the cross-talk between the periphery and central nervous system to regulate energy balance through its actions directed mainly in the hypothalamus. However, leptin receptors are expressed in liver, muscle, adipose tissue and pancreatic β cells, suggesting that it acts directly in those peripheral tissues [17]. Compelling evidence suggests that adipose tissue is not only the main source of leptin but also can respond directly to it. Sustained leptin treatments can eliminate all visible fat in rodents, and restoration of body weight and fat after termination of leptin treatment is delayed, compared to that after food restriction alone [18]. Because of this delay in restoration of body weight, we hypothesized that leptin treatment resulted in actual fat cell loss by apoptosis, a finding that we have documented, particularly after central administration of leptin [6,19–21]. We have proposed that this is a centrally mediated effect of leptin, particularly because the

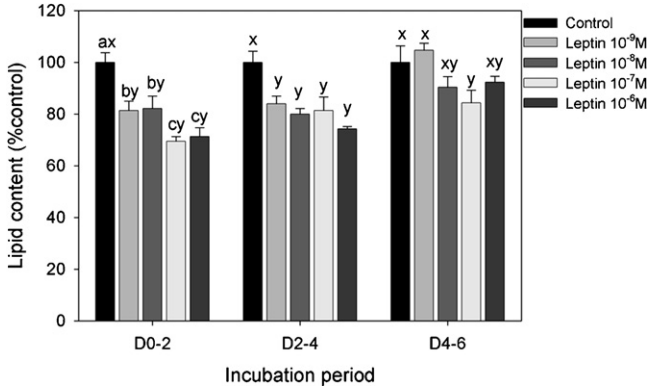

Fig. 3 – Effect of leptin on lipid accumulation (% control) in maturing 3T3-L1 preadipocytes. Cells were treated with leptin (10^{-9}, 10^{-8} and 10^{-7}, 10^{-6} M) for 48 h periods (days 0–2, 2–4 and 4–6) during differentiation. Lipid content was measured on day 6 by AdipoRed assay. Assays were performed in eight replicates for each treatment. Values are means ± S.E.M. Within a time period, means with different letters are different: $^{a,b,c}p < 0.05$; $^{x,y,z}p < 0.01$.

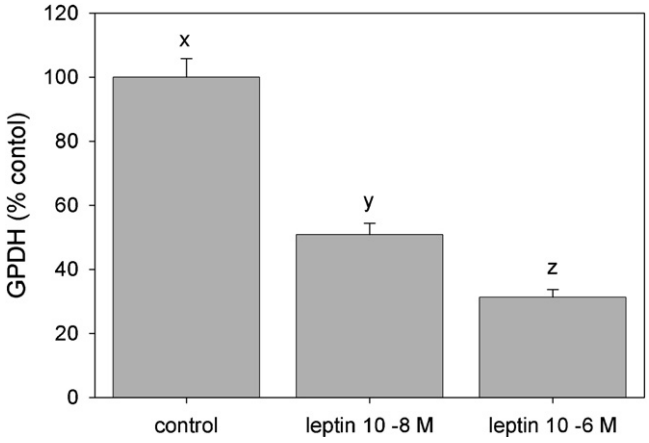

Fig. 5 – Effect of leptin on GPDH activity in maturing preadipocytes (% control). Cells were treated with leptin (10^{-8} and 10^{-6} M) in differentiation media for 6 days (days 0–6). Assays were performed in five replicates for each treatment. Values are means ± S.E.M. Means with different letters (x, y and z) are different, $p < 0.01$.

amounts required centrally are much less than those required with peripheral administration. However, the possibility that leptin can act directly on adipocytes to induce apoptosis has not been tested previously. Therefore, the objective of this study was to determine whether leptin can act directly on adipocytes to induce apoptosis.

The results of this study show that in mouse 3T3-L1 preconfluent preadipocytes, leptin treatment does reduce the number of viable cells, at least in part as a result of increased cell death, as indicated by increased LDH release. Our results are in consonance with the findings of Wagoner et al., whose study in primary rat adipocytes reported similar suppression of preadipocyte proliferation at similar concentrations of leptin [22]. They attributed reduction in cell proliferation to a possible recruitment of different subtypes of the leptin receptor and leptin binding nonspecifically to other cytokine receptors as its

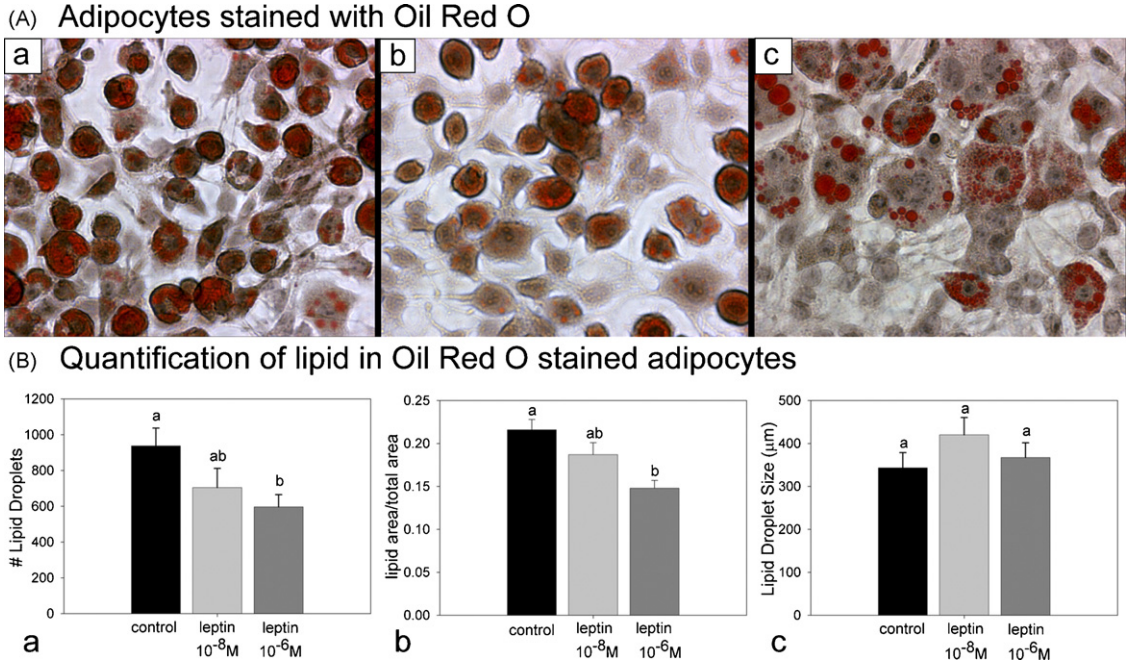

Fig. 4 – Effect of leptin on lipid content and morphology of maturing adipocytes. 3T3-L1 maturing preadipocytes were treated with leptin (10^{-8} and 10^{-6} M) in differentiation media for 6 days (days 0–6). (A) Representative images of Oil-Red-O staining during differentiation ((a) control; (b) leptin 10^{-8} M; (c) leptin 10^{-6} M). (B) Three images for each dish were captured and analyzed. (a) Number of lipid droplets; (b) lipid concentration; (c) mean droplet size. Values are means ± S.E.M. Means with different letters (a and b) are different, $p < 0.05$.

concentration increased as well as regulation of additional circulating factors. This is contrary to the findings of Machinal-Quelin et al., who reported that leptin stimulated proliferation of rat preadipocytes derived from femoral subcutaneous fat [23]. This difference between studies could be ascribed to the use of different cell systems. Whereas the primary culture includes both preadipocytes and fibroblast-like cells, the 3T3-L1 cell line provides a homogenous population with all cells being at the same stage. Immortalized murine cell lines have been established and, owing to their convenience and tractability, have been used widely to study preadipocyte biology [24]. They are relatively accurate representations of true adipocytes, based on ultrastructure and their ability to differentiate and accumulate lipid [25]. This allows investigation of stimuli and mechanism that are associated with regulation of preadipocyte differentiation separately from the ones that regulate differentiation to adipocytes [26–28]. Leptin treatment of maturing preadipocytes also showed similar inhibitory effects on viability, particularly when cells were incubated from days 0–2 and 2–4; the decreased viability was also associated with increased LDH release. Interestingly, similar treatments of leptin in mature adipocytes did not reveal any significant changes in cell viability.

Further, to determine if the decrease in cell number and increased cell death in preconfluent and maturing preadipocytes was the result of apoptosis, ssDNA analysis was performed. Apoptosis is a tightly regulated physiological process, triggered by a variety of metabolic or cytokine-dependent stimuli that leads to cell death [29]. Although we have previously demonstrated that central administration of leptin in rats causes apoptosis of adipose tissue [4], in the present study, leptin did not induce apoptosis in preconfluent preadipocytes, maturing preadipocytes, or in mature adipocytes, thus supporting the hypothesis that leptin acts only centrally to initiate a signal to the adipose tissue to cause apoptosis. Therefore, leptin appears to have its maximal impact on preadipocytes by causing necrotic cell death rather than inducing apoptosis directly. Increase in LDH release also corresponds to a loss of membrane integrity and leakage of lysosomal enzymes, characteristic features of necrosis, but not apoptosis.

Concentrations of leptin used in this study were based on physiological values reported in other studies and from in vivo experiments. Concentrations of approximately 10^{-9} M leptin are in the physiological range [30], although isolated adipocytes from ob/ob mice showed maximal leptin-induced lipolytic effects at 10^{-8} M [26]. In vitro studies examining the effect of leptin on adipocyte metabolism require supra-physiological doses of the protein in order to elicit a influence on either lipolysis or lipogenesis, implying an indirect action of leptin [22]. It is also likely that leptin acts as a paracrine/autocrine agent to affect local adipocytes during which the local leptin concentration could be much higher than that measured in blood. For example, Purdham et al. [31] presented evidence for a paracrine or autocrine effect of leptin in heart muscle. A study by Wang et al. also shows the importance of leptin paracrine effects in the down-regulation of expression of leptin receptor B levels in adipocytes [32].

Adipogenesis is a complex process of differentiation of preadipocytes, involving alteration of cytoskeletal and extracellular matrix proteins, and assembly of the lipid-synthesizing machinery that together generate the mature, rounded and lipid-filled adipocyte phenotype [25]. Data from our study showed that treatment with leptin did suppress lipid accumulation during 3T3-L1 differentiation. This is in agreement with the results using human marrow stromal cells, in which leptin was shown to inhibit differentiation of marrow progenitors to adipocytes and to decrease the accumulation of cytoplasmic lipid droplets, the hallmark of the adipocyte phenotype [33]. We also found that leptin suppressed GPDH activity in maturing preadipocytes, which is generally proportional to triglyceride content of differentiating adipocytes. This is in contrast to findings of Aprath-Husmann et al. [34], who showed a transient increase in GPDH levels in primary human adipocytes.

This study clearly demonstrated that leptin does not act directly to cause adipocyte apoptosis. Our results have shown, however, that leptin functions directly in at least two different ways to inhibit adipocyte development: reduction of preadipocyte viability and inhibition of preadipocyte maturation and lipid storage.

Acknowledgments

This study was supported by the Georgia Research Alliance and the Georgia Research Alliance Eminent Scholar endowment held by CAB.

REFERENCES

[1] Baile CA, Della-Fera MA, Martin RJ. Regulation of metabolism and body fat mass by leptin. Annu Rev Nutr 2000;20:105–27.
[2] Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395:763–70.
[3] Halaas JL, Boozer C, Blair-West J, Fidahusein N, Denton DA, Friedman JM. Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. Proc Natl Acad Sci USA 1997;94:8878–83.
[4] Choi YH, Li C, Page K, Westby A, Della-Fera MA, Lin J, et al. Melanocortin receptors mediate leptin effects on feeding and body weight but not adipose apoptosis. Physiol Behav 2003;79:795–801.
[5] Della-Fera M, Qian H, Baile C. Adipocyte apoptosis in the regulation of body fat mass by leptin. Diab Obes Metab 2001;3:299–310.
[6] Gullicksen PS, Della-Fera MA, Baile CA. Leptin-induced adipose apoptosis: Implications for body weight regulation. Apoptosis 2003;8:327–35.
[7] Qian H, Azain MJ, Compton MM, Hartzell DL, Hausman GJ, Baile CA. Brain administration of leptin causes deletion of adipocytes by apoptosis. Endocrinology 1998;139:791–4.
[8] Prins JB, Walker NI, Winterford CM, Cameron DP. Human adipocyte apoptosis occurs in malignancy. Biochem Biophys Res Commun 1994;205:625–30.
[9] Niesler CU, Siddle K, Prins JB. Human preadipocytes display a depot-specific susceptibility to apoptosis. Diabetes 1998;47:1365–8.
[10] Satoh N, Ogawa Y, Katsuura G, Numata Y, Tsuji T, Hayase M, et al. Sympathetic activation of leptin via the ventromedial hypothalamus: leptin-induced increase in catecholamine secretion. Diabetes 1999;48:1787–93.

[11] Page KA, Hartzell DL, Li C, Westby AL, Della-Fera MA, Azain MJ, et al. Beta-adrenergic receptor agonists increase apoptosis of adipose tissue in mice. Domest Anim Endocrinol 2004;26:23–31.
[12] Hemati N, Ross SE, Erickson RL, Groblewski GE, MacDougald OA. Signaling pathways through which insulin regulates CCAAT/enhancer binding protein alpha (C/EBPalpha) phosphorylation and gene expression in 3T3-L1 adipocytes. Correlation with GLUT4 gene expression. J Biol Chem 1997;272:25913–9.
[13] Frankfurt OS, Krishan A. Identification of apoptotic cells by formamide-induced dna denaturation in condensed chromatin. J Histochem Cytochem 2001;49:369–78.
[14] Frankfurt OS. Immunoassay for single-stranded DNA in apoptotic cells. Methods Mol Biol 2004;282:85–102.
[15] Lin J, Della-Fera MA, Baile CA. Green tea polyphenol epigallocatechin gallate inhibits adipogenesis and induces apoptosis in 3T3-L1 adipocytes. Obes Res 2005;13:982–90.
[16] Wise LS, Green H. Participation of one isozyme of cytosolic glycerophosphate dehydrogenase in the adipose conversion of 3T3 cells. J Biol Chem 1979;254:273–5.
[17] Ceddia RB, Koistinen HA, Zierath JR, Sweeney G. Analysis of paradoxical observations on the association between leptin and insulin resistance. FASEB J 2002;16:1163–76.
[18] Chen G, Koyama K, Yuan X, Lee Y, Zhou YT, O'Doherty R, et al. Disappearance of body fat in normal rats induced by adenovirus-mediated leptin gene therapy. Proc Natl Acad Sci USA 1996;93:14795–9.
[19] Della-Fera MA, Choi Y-H, Hartzell DL, Duan J, Hamrick MW, Baile CA. Sensitivity of ob/ob mice to leptin-induced adipose tissue apoptosis. Obes Res 2005;13:1540–7.
[20] Gullicksen PS, Hausman DB, Hartzell DL, Baile CA. Adipose tissue cellularity and apoptosis after recovery from intracerebroventricular injections of mouse leptin in rats. Obes Res 2001;9:97S.
[21] Hamrick M, Della-Fera MA, Hartzell DL, Choi Y-H, Baile CA. Central control of bone marrow adipocyte populations by leptin. J Bone Miner Res 2005;20:S368.
[22] Wagoner B, Hausman DB, Harris RB. Direct and indirect effects of leptin on preadipocyte proliferation and differentiation. Am J Physiol Regul Integr Comp Physiol 2006;290:R1557–64.
[23] Machinal-Quelin F, Dieudonne MN, Leneveu MC, Pecquery R, Giudicelli Y. Proadipogenic effect of leptin on rat preadipocytes in vitro: activation of MAPK and STAT3 signaling pathways. Am J Physiol Cell Physiol 2002;282:C853–63.
[24] Ailhaud G, Grimaldi P, Negrel R. Cellular and molecular aspects of adipose tissue development. Annu Rev Nutr 1992;12:207–33.
[25] Sorisky A, Gagnon A. Clinical implications of adipose tissue remodelling: adipogenesis and apoptosis. Can J Diab 2002;26:232–40.
[26] Fruhbeck G, Aguado M, Martinez JA. In vitro lipolytic effect of leptin on mouse adipocytes: evidence for a possible autocrine/paracrine role of leptin. Biochem Biophys Res Commun 1997;240:590–4.
[27] Elimam A, Kamel A, Marcus C. In vitro effects of leptin on human adipocyte metabolism. Horm Res 2002;58:88–93.
[28] Ntambi JM, Young-Cheul K. Adipocyte differentiation and gene expression. J Nutr 2000;130:3122S–6.
[29] Kim GS, Hong JS, Kim SW, Koh JM, An CS, Choi JY, et al. Leptin induces apoptosis via ERK/cPLA2/cytochrome c pathway in human bone marrow stromal cells. J Biol Chem 2003;278:21920–9.
[30] Caro JF, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV. Leptin: the tale of an obesity gene. Diabetes 1996;45:1455–62.
[31] Purdham DM, Zou MX, Rajapurohitam V, Karmazyn M. Rat heart is a site of leptin production and action. Am J Physiol Heart Circ Physiol 2004;287:H2877–84.
[32] Wang MY, Orci L, Ravazzola M, Unger RH. Fat storage in adipocytes requires inactivation of leptin's paracrine activity: implications for treatment of human obesity. Proc Natl Acad Sci USA 2005;102:18011–6.
[33] Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL. Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. Endocrinology 1999;140:1630–8.
[34] Aprath-Husmann I, Rohrig K, Gottschling-Zeller H, Skurk T, Scriba D, Birgel M, et al. Effects of leptin on the differentiation and metabolism of human adipocytes. Int J Obes Relat Metab Disord 2001;25:1465–70.