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Interleukin-15 stimulates adiponectin secretion by 3T3-L1 adipocytes: Evidence for a skeletal muscle-to-fat signaling pathway

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Abstract

Interleukin-15 (IL-15) is a cytokine that is highly expressed in skeletal muscle tissue, and that has anabolic effects on skeletal muscle protein dynamics both in vivo and in vitro. Additionally, administration of IL-15 to rats and mice inhibits white adipose tissue deposition. To determine if the action of IL-15 on adipose tissue is direct, the capacity of cultured murine 3T3-L1 preadipocytes and adipocytes to respond to IL-15 was examined. IL-15 administration inhibited lipid accumulation in differentiating 3T3-L1 preadipocytes, and stimulated secretion of the adipocyte-specific hormone adiponectin by differentiated 3T3-L1 adipocytes. The latter observation constitutes the first report of a cytokine or growth factor that stimulates adiponectin production. IL-15 mRNA expression by cultured 3T3-L1 adipogenic cells and C2C12 murine skeletal myogenic cells was also examined. Quantitative real-time PCR indicated IL-15 mRNA was expressed by C2C12 skeletal myogenic cells, and was upregulated more than 10-fold in differentiated skeletal myotubes compared to undifferentiated myoblasts. In contrast, 3T3-L1 cells expressed little or no IL-15 mRNA at either the undifferentiated preadipocyte or differentiated adipocyte stages. These findings provide support for the hypothesis that IL-15 functions in a muscle-to-fat endocrine axis that modulates fat:lean body composition and insulin sensitivity.

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Keywords: Interleukin-15; Adipocytes; Skeletal muscle; Adiponectin; Cytokines

Abbreviations: DMEM, Dulbecco's minimal essential medium; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; IL-15, interleukin-15; IGF-I, insulin-like growth factor-I; MMLV, murine Moloney leukemia virus; PEG, polyethylene glycol; PPAR- γ , peroxisome proliferator-activated receptor-gamma; rh, recombinant human; RIA, radioimmunoassay; RT, reverse transcriptase.

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1. Introduction

The cytokine interleukin-15 (IL-15) is highly expressed at the mRNA level in skeletal muscle tissue (Grabstein et al., 1994). Previous in vitro experiments have demonstrated that IL-15 has anabolic effects on skeletal muscle protein dynamics (Quinn et al., 1995, 2002), conferred by a mechanism which is distinct from that of the well-characterized anabolic factor insulin-like growth factor-I (IGF-I). In addition, IL-15 administration significantly reduces the rate of muscle protein degradation and inhibits skeletal muscle wasting in an in vivo rodent model of cancer cachexia (Carbó et al., 2000). These findings suggest IL-15 may function as an autocrine anabolic mediator of skeletal muscle growth.

In studies investigating the effects of IL-15 on skeletal muscle, it was also observed that IL-15 administration induced 33% reduction in adipose tissue deposition in growing rats, with no reduction in food intake (Carbó et al., 2000, 2001). Such observations cannot distinguish between a direct effect of IL-15 on adipose tissue or an indirect effect on other tissues and/or hormones regulating adipogenesis or energy expenditure. Another in vivo study utilizing obese rodent models (obese Zucker rats and *ob/ob* mice) reported that sensitivity to the fat-inhibiting effects of IL-15 was associated with expression levels of mRNA for a key signaling subunit of the IL-15 receptor in adipose tissue (Alvarez et al., 2002). These data suggest IL-15 reduces adipose tissue mass by a direct mechanism.

The present study was performed to determine if IL-15 has direct effects on a cultured adipogenic cell line. To this end, we examined the effects of recombinant human (rh) IL-15 on lipid deposition and adiponectin secretion (Wolf, 2003) in cultured murine 3T3-L1 cells. We also examined IL-15 mRNA expression by cultured murine 3T3-L1 and C2C12 skeletal myogenic cells. The present data provide evidence to support the involvement of IL-15 in a muscle-to-fat endocrine axis (Carbó et al., 2001), and may contribute to a better understanding of the control of lean/fat body composition, with implications for the pathophysiology and treatment of associated conditions, such as obesity, insulin resistance and type 2 diabetes.

2. Materials and methods

2.1. Cell culture

C2C12 skeletal myogenic and 3T3-L1 adipogenic murine cell lines were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells from both lines were used for experiments at 2–3 passages. For expansion and storage, cell lines were cultured in 10% fetal bovine serum (FBS;

Hyclone, Logan, UT, USA) in high glucose formulation Dulbecco's minimal essential medium (DMEM; Sigma, St. Louis, MO, USA) and cells were stored frozen at -135°C in 20% FBS/DMEM containing 10% dimethylsulfoxide (DMSO; Sigma). For analysis, 3T3-L1 preadipocytes were plated in 1 ml 10% FBS/DMEM in 24-well plates (Costar, Cambridge, MA, USA) at 10^5 cells per well, resulting in confluent cultures by the next day (considered day 0 of culture). To induce differentiation (Engelman et al., 1998), the medium was changed to 10% FBS/DMEM containing 160 nM insulin (Sigma), 0.25 μM dexamethasone (Sigma), and 0.5 mM 3-isobutyl-1-methylxanthine (Sigma). After an additional 48 h (day 2), medium was changed to 10% FBS/DMEM containing 160 nM insulin. Cultures were treated with 0–10 ng/ml rhIL-15 (R&D Systems, Minneapolis, MN, USA) either continuously for 4 days (0–96 h of culture) with replenishment of rhIL-15 at 48 h with the medium change, or for 48 h periods, days 0–2, 2–4, or 4–6 of culture. The latter protocol was utilized to determine the effects of rhIL-15 on lipid deposition and adiponectin secretion at specific periods of adipogenic differentiation, with days 0–2 representing undifferentiated preadipocytes, days 2–4 representing mid-differentiation preadipocytes, and days 4–6 representing differentiated adipocytes.

For analysis of skeletal myogenic cultures, C2C12 myoblasts were plated onto 24-well culture plates at 10^5 cells per well in 10% FBS/DMEM and allowed to attach overnight. To induce differentiation, medium was changed to a lower serum concentration, 0.5% FBS/DMEM, at 24 h after plating (considered day 0). Cells commenced differentiation and fusion into post-mitotic myotubes on day 2, and cultures contained a preponderance of differentiated myotubes by day 5.

2.2. Assay of lipid deposition

Neutral lipid deposition was assessed in 3T3-L1 cultures using a modification of the procedure of Kasturi and Joshi (1982). At specific time points (days 2, 4, or 6), cultures were rinsed once with PBS at 37°C and fixed with 10% formalin in PBS overnight at room temperature. Cultures were rinsed sequentially with dH_2O and 70% ethanol, then stained for 25 min with filtered Oil Red-O solution, 6 parts Oil Red-O-saturated isopropanol (Sigma) in 4 parts dH_2O . Stained cultures were rinsed sequentially with 70% ethanol and dH_2O , and lysed with 4% nonidet P-40 (Calbiochem, La Jolla, CA, USA) in isopropanol on a rotary shaker for 15 min. Absorbance of the dispersed dye at 544 nm was read photometrically using a Victor2 microplate reader (Perkin-Elmer Wallac, Gaithersburg, MD, USA). Results shown represent the mean of three replicates at each time point and/or concentration of rhIL-15, with

differences between control (no IL-15) and experimental treatments determined using Student's *t*-tests.

2.3. DNA assay

DNA content per well was assessed in 3T3-L1 adipogenic cultures by measuring Hoechst 33258 dye (bisbenzimidazole; Sigma) fluorescence as previously described (Haugk et al., 2000). Briefly, cells were rinsed once with DMEM, fixed for 5 min with 70% ethanol/formalin/acetic acid (20:2:1) at 4 °C, then rinsed 3× with DMEM and 2× with PBS. Cells were exposed to 1 µg/ml Hoechst 33258 dye in standard saline citrate for 30 min and analyzed using the fluorescence feature of the Victor2 microplate reader. Results shown represent the mean values for three wells at each concentration of rhIL-15, with differences between control and each concentration of rhIL-15 determined using Student's *t*-tests.

2.4. Adiponectin secretion

Adiponectin was assayed in culture medium collected from differentiating 3T3-L1 adipogenic cultures after 48 h periods of rhIL-15 exposure (0–10 ng/ml), days 0–2, 2–4, or 4–6 of culture in 10% FBS/DMEM, plus the induction factors added according to the schedule outlined above. This protocol was utilized to separate the effects of rhIL-15 on 3T3-L1 differentiation (which could in turn affect adiponectin secretion) from the effects of rhIL-15 on adiponectin secretion at each stage of differentiation. At each time point, medium samples were collected, stored frozen at –20 °C, and thawed immediately prior to analysis. Culture wells from which medium samples were collected were rinsed and used for analysis of lipid deposition as outlined above. Media adiponectin concentrations were determined using a mouse adiponectin radioimmunoassay (RIA) kit (Linco, St. Charles, MO, USA) utilizing ¹²⁵I-labeled murine adiponectin and an anti-adiponectin rabbit antiserum to measure adiponectin concentrations by the double antibody/PEG (polyethylene glycol) technique. Standards over the range of 1–100 ng/ml were prepared using recombinant murine adiponectin. Medium samples were assayed undiluted or diluted 1:4, yielding an effective range of 1–400 ng/ml. The intra-assay and inter-assay coefficients of variation at adiponectin concentrations in the range of 3–12 µg/ml were 3.7–4.4% and 6.6–8.2%, respectively. Medium control samples consisted of 10% FBS/DMEM incubated in 24-well plates with no cells along with the experimental samples. All medium control samples exhibited immunoreactivity of less than 2 ng/ml adiponectin. Results shown represent the mean values for three wells at each concentration of rhIL-15 added to the cultures for each time period, with differences

between control (0 ng/ml IL-15 added) and each concentration of rhIL-15 determined using Student's *t*-tests.

2.5. Quantitative real-time PCR analysis of IL-15 mRNA expression

C2C12 myogenic cultures and 3T3-L1 adipogenic cultures were established and induced to differentiate as described above. RNA was harvested at the undifferentiated preadipocyte (day 0) or myoblast (day 0) stages, mid-differentiation stages (day 2 for 3T3-L1 and day 3 for C2C12), and differentiated adipocyte or myotube stages (day 4 for 3T3-L1 and day 5 for C2C12) of culture. Total RNA was isolated and purified from C2C12 and 3T3-L1 cultures using the RNeasy Fibrous Tissue or Lipid Tissue Kits (Qiagen, Valencia, CA, USA), respectively. RNA samples were treated on column with 30 Kunitz units DNase I (Qiagen) to remove residual genomic DNA, then quantified using the RiboGreen RNA Quantitation Kit (Molecular Probes, Eugene, OR, USA). cDNA was reverse transcribed from 1 µg total RNA using the Bio-Rad iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA).

Quantitative real-time polymerase chain reaction (PCR) analysis of IL-15 mRNA expression was performed using a Bio-Rad iCycler, and IL-15 mRNA expression normalized to 18S ribosomal RNA expression for each sample. Total RNA (1 µg) was run in 20 µl reactions using a blend of oligo dT and random hexamer primers and a modified MMLV-derived reverse transcriptase (RT; Bio-Rad). Reaction cycles were 5 min at 25 °C, 30 min at 42 °C, and 5 min at 85 °C. Each cDNA reaction was diluted 5×, along with a calibrator sample containing the transcript of interest, and run in 50 µl Bio-Rad SYBR Green reactions using 200 µM primers designed by Beacon Designer software (BioSoft International, Palo Alto, CA, USA). Primers for murine IL-15 mRNA, which detected either of two RNA splice variants coding for identical mature IL-15 peptides (Fehniger and Caligiuri, 2001), were: forward 5'-CAG CAC TCT GTC TTC TAA CAA GAA-3'; reverse 5'-TCT GTG AAG GTT TTC TCC TCC A-3'. Primers for 18S ribosomal RNA, used as the "housekeeping" gene, were: forward 5'-GCG AAT GGC TCA TTA AAT CAG TTA-3'; reverse 5'-TGG TTT TGA TCT GAT AAA TGC ACG-3'.

Reactions were processed using one initial denaturation cycle (5 min at 95 °C), then 40 cycles of denaturation (30 s at 95 °C), annealing (30 s at 61 °C) and amplification (30 s at 72 °C) followed by melt curve determination consisting of one denaturation cycle (1 min at 95 °C), annealing (one cycle for 1 min at 62 °C), then 68 cycles (10 s each at 61 °C to 95 °C). The 18S ribosomal RNA primer pair reaction was run on every sample for template content normalization

purposes. A calibrator RT sample containing the cDNA of interest was run to yield a standard curve for each primer set, and individual primer reaction efficiencies were calculated from this curve using the iCycler software. The iCycler software calculated a threshold cycle for each sample; threshold cycles and primer pair efficiencies were used in the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001) to yield differences in IL-15 mRNA expression as normalized to the 18S housekeeping gene.

3. Results

3.1. Effects of IL-15 on lipid deposition in 3T3-L1 adipogenic cultures

Incubation of 3T3-L1 cells with 2–10 ng/ml rhIL-15 from days 0–4 of culture resulted in approximately 20% decreases ($p < 0.001$, t -tests) in Oil Red-O staining compared to cultures treated with vehicle alone (Fig. 1A). DNA content per well did not differ among treatment groups (Fig. 1B), indicating the decrease in lipid deposition was not an artifact of reduced cell numbers due to cytokine toxicity. The effects of rhIL-15 on 3T3-L1 lipid deposition at specific periods of culture, representing the progression from undifferentiated preadipocytes (days 0–2) to mid-differentiation preadipocytes (days 2–4) to differentiated adipocytes (days 4–6), were also determined (Fig. 1C). When 3T3-L1 cultures were treated with rhIL-15 from days 0 to 2, Oil Red-O staining was reduced more than 50% ($p < 0.05$). In contrast, rhIL-15 treatment of cultures from days 2 to 4 resulted in only a 15% inhibition of lipid deposition, which was nevertheless significant ($p < 0.05$). Lipid deposition rose dramatically between days 4 and 6. However, rhIL-15 exposure from days 4 to 6 did not result in a significant decrease in Oil Red-O staining, indicating IL-15 does not inhibit lipid deposition by differentiated 3T3-L1 adipocytes.

3.2. Effects of IL-15 on adiponectin secretion in 3T3-L1 adipogenic cultures

To determine if IL-15 could modulate the endocrine function of differentiated adipocytes, the effects of rhIL-15 treatment on secretion of adiponectin were determined by RIA of culture medium (Fig. 2). Adiponectin concentrations in medium collected from cultures during days 0–2 were equivalent to those in medium control samples (incubated without cells), indicating 3T3-L1 preadipocytes secrete undetectable amounts of adiponectin (not shown). Treatment of preadipocytes with rhIL-15 on days 0–2 had no effect on adiponectin secretion (Fig. 2A). Adiponectin secretion from day 2–4 cultures was low but detectable, and was also unaffected

Fig. 1. Effects of rhIL-15 on lipid deposition in differentiating 3T3-L1 preadipocyte cultures. (A) Lipid deposition assessed for 4 days following induction of adipocyte differentiation; cultures were exposed continuously to 0–10 ng/ml rhIL-15. (B) DNA content per well assessed for 4 days following induction of adipocyte differentiation; cultures were exposed continuously to 0–10 ng/ml rhIL-15. (C) Effects of rhIL-15 administered at specific 48 h periods in culture. Exposure periods (0–2, 2–4, 4–6) indicate days of culture following addition of inducers, ± 2 ng/ml rhIL-15, followed by processing of cultures at days 2, 4, or 6, respectively. Lipid deposition was assessed by Oil Red-O staining, assayed photometrically; DNA content was assessed by Hoechst 33258 dye fluorescence. For all panels, each bar represents the mean $\pm$ SEM of three replicates, with differences between control (no rhIL-15) and rhIL-15-treated cultures determined by t -tests (* $p < 0.05$; *** $p < 0.001$).

by rhIL-15 treatment (Fig. 2A). Adiponectin secretion increased dramatically by days 4–6, coincident with adipocyte differentiation (Fig. 2A). Treatment of day 4–6 cultures with 10 ng/ml rhIL-15 significantly increased adiponectin secretion from 71.5 ± 3.8 ng/ml to

Fig. 2. Effects of IL-15 on adiponectin secretion in 3T3-L1 adipogenic cultures. (A) Effects of 10 ng/ml rhIL-15 administered at specific 48 h periods in culture. Exposure periods (0–2, 2–4, 4–6) indicate days of culture following addition of inducers, $\pm$ 10 ng/ml rhIL-15, followed by processing of cultures at days 2, 4, or 6, respectively. (B) Adiponectin secretion by 3T3-L1 adipocytes exposed to 0–10 ng/ml rhIL-15 on days 4–6 following induction of adipocyte differentiation. (C) Lipid content of 3T3-L1 adipocytes exposed to 0–10 ng/ml rhIL-15 on days 4–6 following induction of adipocyte differentiation. Adiponectin secretion was determined by RIA of culture medium; lipid deposition was determined by microplate-based assay of oil Red-O staining. For all panels, each bar represents the mean $\pm$ SEM of three replicates, with differences between control (no rhIL-15) and rhIL-15-treated cultures determined by *t*-tests (* p < 0.05; *** p < 0.001).

128.8 ± 4.0 ng/ml (p < 0.001). The effect of rhIL-15 on adiponectin secretion by 3T3-L1 adipocytes was concentration-dependent with a threshold of 5 ng/ml (Fig. 2B), while lipid content in adipocyte cultures treated with rhIL-15 on days 4–6 was unchanged (Fig. 2C).

3.3. Expression of IL-15 mRNA in cultured skeletal myogenic and adipogenic cells

IL-15 mRNA expression in cultured skeletal myogenic and adipogenic cells has not been characterized with respect to differentiation stages. Fig. 3 shows real-time quantitative PCR analysis of IL-15 mRNA expression by cultured 3T3-L1 adipogenic cells and C2C12 skeletal myogenic cells. For both cell types, IL-15 mRNA expression was assessed at the undifferentiated precursor cell (preadipocyte, myoblast) stages, at mid-differentiation, and at the differentiated cell (adipocyte, myotube) stages. C2C12 myoblasts (culture day 0) expressed IL-15 mRNA which was detectable after 32.5 cycles of PCR. By the mid-differentiation and differentiated myotube stages (days 3–5 of culture), IL-15 mRNA expression increased more than 10-fold, and was significantly different from the level of IL-15 mRNA expression in undifferentiated myoblast cultures (p < 0.01, *t*-tests; not indicated in Fig. 3). In contrast, IL-15 mRNA expression by 3T3-L1 cells was detected at 34.7–38 cycles of PCR, equivalent to background levels and significantly lower than the levels detected in C2C12 cells at equivalent stages of differentiation (p < 0.01, *t*-tests). These findings indicate cultured skeletal myogenic cells express IL-15 mRNA which is upregulated concurrent with differentiation into myotubes, whereas cultured 3T3-L1 preadipocytes and adipocytes express little or no IL-15 mRNA under these conditions.

Fig. 3. Expression of IL-15 mRNA by cultured mouse 3T3-L1 adipogenic and C2C12 skeletal myogenic cell lines, assessed by quantitative real-time PCR. For 3T3-L1 cultures, RNA was harvested at the undifferentiated preadipocyte (day 0), mid-differentiation (day 2), and differentiated adipocyte (day 4) stages of culture. For C2C12 cultures, RNA was harvested at the undifferentiated myoblast (day 0), mid-differentiation (day 3), and differentiated myotube (day 5) stages of culture. Each bar represents the mean $\pm$ SEM of three replicates, with differences between 3T3-L1 and C2C12 cultures at each stage determined by *t*-tests (** p < 0.01; *** p < 0.001). IL-15 mRNA expression by C2C12 cultures also differed significantly between undifferentiated and mid-differentiated stages (p < 0.001, *t*-test), and between undifferentiated and differentiated stages (p < 0.01), not indicated by asterisks in the figure.

4. Discussion

Previous *in vivo* studies suggested that IL-15 inhibits fat deposition via a direct action on adipose tissue in rodents (Alvarez et al., 2002). The present study utilized the murine 3T3-L1 preadipocyte cell line to investigate the direct action of IL-15 on cultured adipogenic cells. Incubation of 3T3-L1 preadipocytes with rhIL-15 inhibited lipid deposition by more than 50%, an effect which decreased as differentiation progressed. IL-15 had no effect on lipid content in differentiated 3T3-L1 adipocytes; however, IL-15 instead acted to stimulate secretion of the adipocyte-specific hormone, adiponectin, by these cells. These observations demonstrate IL-15 can exert direct actions on 3T3-L1 cells at both the preadipocyte and differentiated adipocyte stages.

This study also examined IL-15 mRNA expression by cultured mouse adipogenic and skeletal myogenic cell lines. The results are in agreement with previous *in vivo* studies reporting high expression of IL-15 mRNA by skeletal muscle tissue (Grabstein et al., 1994). Furthermore, IL-15 mRNA expression by a determined skeletal myogenic cell line and its upregulation during differentiation, suggests that muscle cells themselves, as opposed to intramuscular vascular, connective tissue, or immune infiltrating cells (Weisberg et al., 2003; Xu et al., 2003), express IL-15 mRNA. In contrast, we observed little or no IL-15 mRNA expression by cultured 3T3-L1 cells, at any stage of differentiation, under the conditions tested. Our results do not exclude the possibility of IL-15 production by adipocytes or adipose tissue under other conditions. Indeed, a recent report indicated explanted porcine adipocytes express IL-15 mRNA when stimulated by interferon-gamma (Ajuwon et al., 2004). However, our findings demonstrate vastly different basal levels of IL-15 mRNA expression between cultured adipocyte and muscle cell lines. Differences in IL-15 expression levels between adipocyte cell lines and explanted primary adipocytes could be due to the presence of macrophage-like infiltrating cells in the primary cultures (Weisberg et al., 2003; Xu et al., 2003).

In addition to its primary role as an energy storage depot, the role of adipose tissue as an endocrine organ is increasingly recognized (Wolf, 2003; Steppan and Lazar, 2002; Sonnenberg et al., 2004; Havel, 2004). Adipose tissue secretes a number of biologically active factors, termed adipocytokines, which include adiponectin, leptin, tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and resistin (Wolf, 2003; Steppan and Lazar, 2002; Sonnenberg et al., 2004; Havel, 2004). These secreted proteins can act directly on peripheral tissues, such as skeletal muscle, to regulate processes such as protein turnover and insulin sensitivity (Fig. 4, upper panel), and can also act by regulating central nervous tissue pathways which regulate appetite, feeding behavior, and energy expenditure (Wolf, 2003; Steppan

Fig. 4. Models for peripheral control of fat and muscle. (A) Previous model based on studies showing growth factors/hormones released from white adipose tissue affect skeletal muscle. (B) New model, originally proposed by Carbó et al. (2001), suggesting muscle-derived IL-15 may play a role in modulating white adipose tissue mass and/or function.

and Lazar, 2002; Sonnenberg et al., 2004; Havel, 2004). The results of our study support the model originally proposed by Carbó et al. (2001), suggesting that muscle tissue may in turn secrete IL-15, which acts to inhibit adipose tissue deposition (Fig. 4, lower panel). The present study provides evidence of a direct effect of IL-15 on preadipocytes and adipocytes, and further indicates that IL-15 can modulate adipose tissue endocrine function, e.g. adiponectin secretion.

Further confirmation of the proposed model of a muscle-to-fat endocrine axis will require *in vivo* studies and examination of IL-15 protein secretion patterns. The patterns of IL-15 mRNA expression by cultured adipogenic and skeletal myogenic cell lines observed in the present study support this model. Moreover, multiple tissue Northern blots indicated skeletal muscle and placenta are the major sites of IL-15 mRNA expression *in vivo* (Grabstein et al., 1994). The IL-15 mRNA isoform expressed in skeletal muscle is the form believed to be more readily secreted (Fehniger and Caligiuri, 2001). However, it is not yet known whether adipose tissue *in vivo* is exposed to biologically relevant concentrations of IL-15 produced by muscle. Recent studies of mice which carried a muscle-specific deletion of the peroxisome proliferator-activated receptor- γ (PPAR γ) gene suggested this deletion indirectly affected secretion of an unidentified, muscle-derived hormone which in turn affected adipose tissue (Hevener et al., 2003). Our findings suggest IL-15 could be this putative factor.

IL-15 mRNA is widely expressed at lower levels in a number of non-lymphoid tissues, including heart, lung, liver, kidney, brain, and testes (Fehniger and Caligiuri, 2001). Along with interleukin-2, an important role for IL-15 is to support the development of several types of T lymphocytes, particularly natural killer and CD8+ ("memory T") cells (Fehniger and Caligiuri, 2001). Due to these actions, IL-15 inhibits lethality in murine models of infectious diseases such as retrovirus-induced acquired immunodeficiency syndrome (Umemura et al., 2002) and herpes simplex virus infection (Tsunobuchi et al., 2000). Additionally, IL-15 has proinflammatory activity in several mouse models of autoimmune diseases, such as rheumatoid arthritis and inflammatory bowel disease (McInnes et al., 1997; Vainer et al., 2000). However, in other systems, such as a murine model of nephritis, IL-15 exerts anti-inflammatory activity (Shinozaki et al., 2002). In several in vitro and in vivo systems, IL-15 inhibits the proinflammatory and pro-apoptotic actions of TNF- α (e.g., Bulfone-Paus et al., 1999; Hiromatsu et al., 2003).

Increased production of the proinflammatory adipocytokines IL-6 and TNF- α during obesity has been mechanistically linked to insulin resistance (Weisberg et al., 2003; Xu et al., 2003; Ajuwon et al., 2004; Sonnenberg et al., 2004; Lyon et al., 2003). TNF- α and IL-6 inhibit adiponectin mRNA and protein secretion (Havel, 2004; Maeda et al., 2001; Fasshauer et al., 2003), while the present study showed IL-15 stimulates adiponectin secretion from cultured adipocytes. In skeletal muscle, IL-6 and TNF- α exert catabolic actions, inhibiting skeletal myoblast differentiation and stimulating muscle fiber protein degradation (Reid and Li, 2001; Tsujinaka et al., 1995). In contrast, IL-15 exerts anabolic actions in skeletal muscle in vivo and in vitro via inhibition of muscle fiber protein degradation (Quinn et al., 2002; Carbó et al., 2000). The opposing actions of IL-15 and TNF- α on muscle protein degradation and adiponectin secretion by adipocytes suggests that IL-15 may function to inhibit or oppose TNF- α -mediated pathways in both skeletal muscle and adipose tissue.

An important finding of this study was the stimulation of adiponectin secretion observed during incubation of differentiated 3T3-L1 cells in the presence of IL-15. Adiponectin is a secreted protein produced exclusively by adipocytes in most physiological conditions (Havel, 2004; Pajvani and Scherer, 2003; Delaigle et al., 2004). Circulating levels of adiponectin are negatively correlated with visceral adiposity and insulin resistance in both rodents and humans (Wolf, 2003; Sonnenberg et al., 2004; Havel, 2004; Weyer et al., 2001; Cnop et al., 2003). Adiponectin exerts actions on both liver and skeletal muscle, where it activates AMP-activated protein kinase and stimulates fatty acid oxidation, resulting in improved insulin sensitivity (Yamauchi et al., 2001,

2002). Adiponectin may also have actions in the central nervous system to increase energy expenditure (Qi et al., 2004). In addition, adiponectin has direct anti-inflammatory actions on vascular endothelium and may protect against the progression of atherosclerosis (Havel, 2004; Yamauchi et al., 2003).

Due to the known insulin-sensitizing and anti-atherosclerotic actions of adiponectin, the regulation of adiponectin expression and secretion is of considerable interest. The insulin-sensitizing thiazolidinedione class of PPAR γ agonists appear to function in part by stimulating adiponectin expression (Sonnenberg et al., 2004; Havel, 2004; Maeda et al., 2001). The observation reported here that IL-15 stimulates adiponectin secretion by cultured 3T3-L1 adipocytes represents the first report of an endogenous cytokine or growth factor which stimulates adiponectin secretion. These data suggest that IL-15 could potentially act in vivo to stimulate adiponectin production by adipose tissue, which would in turn modulate skeletal muscle metabolism and insulin sensitivity.

The present data demonstrating that IL-15 has direct effects on cultured 3T3-L1 preadipocytes and adipocytes provides evidence supporting the involvement of IL-15 in a muscle-to-fat endocrine axis (Carbó et al., 2001). Further investigation of this hypothesis is likely to provide a better understanding of the control of body composition and substrate metabolism, with implications for the pathophysiology and treatment of associated conditions such as obesity, insulin resistance, and type 2 diabetes.

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