Direct regulation of lipolysis by interleukin-15 in primary pig adipocytes

Kolapo M. Ajuwon and Michael E. Spurlock

Department of Animal Sciences, Comparative Medicine Program, Purdue University, West Lafayette, Indiana 47907-2054

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Ajuwon, Kolapo M., and Michael E. Spurlock. Direct regulation of lipolysis by interleukin-15 in primary pig adipocytes. Am J Physiol Regul Integr Comp Physiol 287: R608–R611, 2004. First published May 20, 2004; 10.1152/ajpregu.00192.2004.—We recently provided evidence that interleukin-15 (IL-15) is expressed lowly in the pig adipocyte and that interferon-γ (IFN-γ) markedly increases this expression through a pathway regulated in part by protein kinase C (Ajuwon KM, Jacobi SK, Kuske JL, and Spurlock ME. Am J Physiol Regul Integr Comp Physiol 286: R547–R553, 2004). In the present study, we tested the hypothesis that IL-15 acts directly on the adipocyte to regulate lipid accretion by enhancing lipolysis or suppressing lipogenesis. Using recombinant porcine IL-15, we determined that this cytokine stimulates lipolysis in a dose-dependent manner (P < 0.001). Furthermore, comparative studies with other cytokines showed that IL-15 is more potent in its acute stimulation of lipolysis than either TNF-α, IL-6, or LPS (P < 0.001). When specific inhibitors of protein kinase A or Janus kinase are present, the lipolytic effect of IL-15 is attenuated (P < 0.01). These data indicate that, in addition to its regulation of muscle protein accretion and T-cell growth and development, IL-15 also targets the adipocyte directly to alter stimulate lipolysis. Thus, when induced by IFN-γ or other inflammatory mediators, IL-15 may be a significant homeostatic factor that mobilizes and directs energy away from the adipocyte to other cells during the acute phase of the inflammatory response.

INTERLEUKIN-15 (IL-15) is highly expressed in skeletal muscle and has been shown to exert a powerful anabolic effect on muscle protein accretion (16, 17). In addition to its enhancement of muscle growth, there is also evidence that IL-15 suppresses fat accretion in rodent models, perhaps through a direct effect on the adipocyte. Recently, Alvarez et al. (3) provided conclusive evidence of the expression of the IL-15 receptor in adipose tissue of rodents. Furthermore, the reduction in adiposity achieved with IL-15 in lean Zucker rats was precluded in fa/fa rats in which adipose expression of the γ(c) signaling receptor subunit is also markedly lower than in lean controls. Consequently, these investigators suggested that the effect of IL-15 on adipose mass was indeed a direct effect on the adipocyte.

We have recently demonstrated that IL-15 expression is strongly upregulated in primary porcine adipocytes treated with IFN-γ (2). Because the latter cytokine is a major inflammatory mediator (8, 24), it is plausible that its induction of IL-15 is a component of the acute-phase response that facilitates the mobilization and repartitioning of energy away from the adipocyte to support other immediate needs, albeit there is currently no evidence that IL-15 acts directly on the adipocyte to alter lipogenesis or lipolysis, the major processes by which lipid storage and release in the adipocyte are regulated. Consequently, the experiments reported herein were designed to test the hypothesis that IL-15 acts directly on the adipocyte to enhance lipolysis and suppress lipogenesis. Additionally, we sought to identify specific steps in the pathways by which IL-15 exerts these effects.

MATERIALS AND METHODS

Adipocyte isolation. Adipocytes from subcutaneous fat depot of castrate male pigs weighing ~100 kg were isolated by collagenase digestion as described previously (2). The isolated cells were diluted to approximate 20% cell suspensions with DMEM (Sigma, St. Louis, MO) containing 3% fatty acid-free serum albumin. The cell suspensions were gassed initially and at 2-h intervals with a mixture of air and CO2 and were incubated in a gyratory floor incubator at 37°C for the selected duration. Reactions were terminated by allowing adipocytes to float on the surface and carefully aspirating the medium from the bottom of the vials. The medium was assayed for glycerol using the Triglyceride Reagent from Sigma, and data are expressed as micrograms per milliliter. The rate of lipogenesis was determined as the incorporation of [U-14C]glucose into fatty acids as described by Liu et al. (14). Briefly, cells were incubated for 2 h in the presence of 0.5 μCi of [14C]glucose. Samples were processed as described previously, and rates are expressed as picomoles per minute.

To study intracellular kinases involved in IL-15 action, adipocytes were pretreated for 1–2 h with the selective enzyme inhibitors before the addition of IL-15 (Biosource, Camarillo, CA) to the medium. Inhibitors of MEK (U-0126), protein kinase C (PKC) (bisindolylmaleimide HCl), Janus kinase (JAK)-STAT (AG-490), protein kinase A (PKA) (H-89), and phosphatidylinositol-3 kinase (PI3K) (wortmannin) were obtained from Calbiochem (La Jolla, CA). U-0126 and bisindolylmaleimide HCl were used at 15 and 20 μM, respectively. Wortmannin, H-89, and AG-490 were used at concentrations of 1, 20, and 100 μM, respectively. All experiments were repeated at least three times with cells obtained from different pigs. Within each experiment (pig), each treatment was replicated at least three times.

Statistical analyses. All data were examined for normality and analyzed with the mixed-model analysis of a split-plot design. The fixed effect was the treatment, and the random effect was the shade. The main effects (treatment and replicate) were tested against the treatment × replicate interaction. When protected by a significant F-test, mean separation was accomplished using the least-squares mean separation (PDIFF) procedure (20).

RESULTS

IL-15 stimulates lipolysis in a dose- and time-sensitive manner. As presented in Fig. 1, IL-15 stimulated lipolysis (glycerol release) in a dose-dependent manner, with little stimulation at 25 ng/ml but a significant twofold induction at 50 ng/ml and a threefold induction at 100 ng/ml (P < 0.001). Additionally, the effect of IL-15 was time sensitive (P < 0.001), with the greatest stimulation at 2 h, and a rapid diminution thereafter (Fig. 2). Compared with other proinflam-
matory cytokines, the acute potency of IL-15 is markedly higher (Fig. 3). Whereas the lipolytic effect of IL-15 was readily apparent after 2 h \((P < 0.001)\), that of other potentially lipolytic cytokines (TNF-\(\alpha\), IL-6) was not, nor was there an effect of bacterial LPS.

**Activation of PKA and JAK mediates the lipolytic effect of IL-15.** To identify critical intracellular signaling arms for the lipolytic effect of IL-15, we used an array of selective inhibitors that have been documented to mediate IL-15 signal transduction in other cell types. As shown in Fig. 4A, inhibition of PKA and JAK by H-89 and AG-490, respectively, attenuated the ability of IL-15 to enhance lipolysis \((P < 0.001)\). In contrast, inhibitors of MEK, PKC, and PI3K (U-0126, bisindolylmaleimide, and wortmannin, respectively) did not alter the efficacy of IL-15 as a lipolytic effector (Fig. 4B).

**Inhibition of lipogenesis by IL-15.** To establish whether the regulatory actions of IL-15 also include lipogenesis, we tested the effect of this cytokine on the incorporation of glucose into adipocytes treated with 25, 50, or 100 ng/ml IL-15 for 2 h, and the concentration of glycerol in the medium was determined. IL-15 stimulates significant glycerol release at 50 and 100 ng/ml. Data are means ± SE from 3 different pigs. *Significance at \(P < 0.001\).

Fig. 1. IL-15 induces lipolysis (glycerol release) in pig adipocytes. Adipocytes were treated with buffer or 25, 50, or 100 ng/ml IL-15 for 2 h, and the concentration of glycerol in the medium was determined. IL-15 stimulates significant glycerol release at 50 and 100 ng/ml. Data are means ± SE from 3 different pigs. *Significance at \(P < 0.001\).

Fig. 2. Kinetics of glycerol release and accumulation in the media of adipocytes treated with IL-15. Adipocytes were treated with buffer (-) or 50 ng/ml IL-15 (+) for 2, 4, 6, and 8 h, and the concentration of glycerol in the medium was determined. IL-15-stimulated glycerol release was maximal at 2 h and declined thereafter. Data are means ± SE from 3 different pigs. *Significant difference from cells treated with buffer at \(P < 0.001\). Treatment × time, \(P < 0.001\).

Fig. 3. Comparison of the lipolytic effect of IL-15 vs. TNF-\(\alpha\), IL-6, and LPS. Adipocytes were treated with IL-15, TNF-\(\alpha\), and IL-6 (50 ng/ml) or LPS (10 \(\mu\)g/ml) for 2 h. Only IL-15 caused significant glycerol release within the 2-h incubation. Data are means ± SE from 3 different pigs. *Significant difference from basal [control (C)] at \(P < 0.001\).

Fig. 4. A: IL-15-induced lipolysis is inhibited by H-89 and AG-490, inhibitors of protein kinase A (PKA) and Janus kinase (JAK), respectively. Adipocytes were cultured for 2 h with the inhibitors before being treated with IL-15 for an additional 2 h. Lipolysis was inhibited in the presence of H-89 and AG-490. Data are means ± SE from 3 different pigs. Means with different superscript lowercase letters differ at \(P < 0.001\). B: inhibition of MEK, PKC, and phosphatidylinositol-3 kinase (PI3K) [by U-0126, bisindolylmaleimide HCl (Bis), and wortmannin (Wort), respectively] did not inhibit the lipolytic effect of IL-15. Adipocytes were cultured in the presence of U-0126, Bis, and Wort for 2 h before IL-15 was added for an additional 2 h. Data are means ± SE from 3 different pigs.
lipid. IL-15 significantly \((P < 0.05)\) reduced the incorporation of labeled glucose into extractable lipid, but the magnitude of the suppression was only 5–7% vs. controls (data not shown).

**DISCUSSION**

IL-15 is a 14-kDa member of the 4α helix bundle family of cytokines, which communicate immunological needs to T-cell and natural killer cell populations (18, 19). Although a clear participant in immune modulation cascades, this cytokine is highly expressed in skeletal muscle (21, 22), where it promotes protein accretion (16) and antagonizes muscle wasting in some disease models (7). We recently demonstrated that low-level IL-15 expression is markedly upregulated in pig adipocytes treated with IFN-γ (2), the latter cytokine being a major mediator of inflammation in pigs (8). Based on this finding, and on the indications that IL-15 might act directly on the adipocyte to regulate fat accretion (3), we hypothesized that IL-15 would enhance lipolysis and suppress lipogenesis. Indeed, the evidence presented indicates that IL-15 does target the adipocyte directly, and in an acute manner, to transiently stimulate lipolysis and suppress lipogenesis. The concentrations of IL-15 used in our experiments are higher than in the systemic circulation (9, 11), but secretion of IL-15 by the adipocyte may present these cells with substantially higher concentrations than is reflected in the circulation. The antilipogenic effect of IL-15 was quite small, and its biological significance is unclear. The small attenuation achieved after 2 h may have a greater impact in vivo, but longer term experiments will be required to address this issue.

Collectively, these findings indicate a potential autocrine regulatory axis in which IL-15 is induced by IFN-γ, with the metabolic effects of mobilizing fatty acids for use as energy or as precursors to signaling molecules such as diacyl glycerol or phosphoinositides. Although it will be necessary to confirm that the IL-15 protein is actually synthesized and released by the adipocyte before the evidence of such an autocrine loop will be conclusive, it is already apparent that serum IL-15 in many inflammatory states (9, 11). Thus the adipocyte may contribute to the increased circulating concentrations.

The transient nature of the increase in media glycerol concentrations caused by IL-15 is perplexing. The data indicate that a net disappearance of glycerol from the media ensues between 2 and 4 h of incubation, despite the continued presence of IL-15. Free glycerol and some monoacyl glycerol esters readily traverse the adipocyte membrane (10, 12, 13). In the pig adipocyte, inhibition of JAK with AG-490 attenuates the lipolytic action of IL-15, as does inhibition of PKA.

Lipolysis in the adipocyte is mainly driven by hormone-sensitive lipase, which is activated through phosphorylation by PKA. Therefore, it is not surprising that IL-15 would stimulate lipolysis via mechanisms that involve PKA. However, in view of the appreciable difference in the magnitude of the inhibition of IL-15-induced lipolysis by blockade of PKA vs. that achieved by inhibition of JAK, PKA activation is unlikely a major determinant of IL-15-induced lipolysis.

In summary, we have extended our initial finding that IL-15 is inducible in the primary pig adipocyte by IFN-γ to show that the adipocyte is highly responsive to this cytokine in terms of enhanced lipolysis, and to a lesser extent, suppressed lipogenesis. These data mark the first conclusive evidence that lipolysis and lipogenesis are directly targeted by IL-15 in any adipocyte model. Furthermore, we have identified the JAK and PKA pathways as partial mediators of the lipolytic effect of IL-15. These data point to a likely role for IL-15 in the alterations of lipid metabolism and energy partitioning that accompany the acute inflammatory response and infection.

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**REFERENCES**


