Domestic Animal Endocrinology 61 (2017) 62–76

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.domesticanimalendo.com

Adipose triglyceride lipase protein abundance and translocation to the lipid droplet increase during leptin-induced lipolysis in bovine adipocytes

D.A. Koltes [a,*,†], M.E. Spurlock [b], D.M. Spurlock [a]

[a] Department of Animal Science, Iowa State University, Ames, Iowa 50011, USA
[b] Department of Food Science and Human Nutrition, Iowa State University, Ames, Iowa 50011, USA

ARTICLE INFO

Article history:
Received 17 January 2017
Received in revised form 30 May 2017
Accepted 2 June 2017

Keywords:
Glycerol
Hormone sensitive lipase
Signal transducer and activator of transcription 3
Leptin

ABSTRACT

Proper regulation of lipid metabolism is critical for preventing the development of metabolic diseases. It is clear that leptin plays a critical role in the regulation of energy homeostasis by regulating energy intake. However, leptin can also regulate energy homeostasis by inducing lipolysis in adipocytes, but it is unclear how the major lipases are involved in leptin-stimulated lipolysis. Therefore, the objectives of this study were to determine if (1) leptin acts directly to induce lipolysis in bovine adipocytes, (2) the potential lipases involved in leptin-induced lipolysis in bovine adipocytes, and (3) increases translocation of adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL) during leptin-stimulated lipolysis in bovine stromal vascular cell–derived adipocytes. As hypothesized, leptin induced a lipolytic response ($P = 0.02$) in isolated adipocytes which was accompanied by an increase in phosphorylation of signal transducer and activator of transcription (STAT)3 ($P = 0.03$), a well-documented secondary messenger of leptin, and ATGL protein abundance ($P < 0.01$). Protein abundance of STAT3, perilipin, HSL, and phosphorylation of HSL by PKA and AMPK were not altered during leptin-stimulated lipolysis ($P > 0.05$). Immunostaining techniques were employed to determine the location of HSL and ATGL. Both lipases translocated to the lipid droplet after 2 h of exposure to isoproterenol ($P < 0.02$). However, only ATGL was translocated to the lipid droplet during leptin-stimulated lipolysis ($P = 0.04$), indicating ATGL may be the active lipase in leptin-stimulated lipolysis. In summary, leptin stimulates lipolysis in bovine adipocytes. The lack of phosphorylated HSL and translocation of HSL to the lipid droplet during leptin-stimulated lipolysis suggest minimal activity by PKA. Interestingly, leptin-stimulated lipolysis is accompanied by an increase in ATGL protein abundance and translocation to the lipid droplet, indicating its involvement in leptin-stimulated lipolysis either due to an increase in protein abundance or through a novel lipolytic cascade.

1. Introduction

Lipolysis is a key component of lipid metabolism and energy homeostasis. This is readily apparent in lactating dairy cattle because energy requirements for milk production exceed energy intake, thus necessitating the mobilization of fatty acids from adipose tissue. When lipolysis is acutely upregulated or prolonged for extended periods of time in over-conditioned cows, it increases the risk of ketosis, fatty liver disease, displaced abomasum, dystocia, retained placenta, lower milk production, and reduced reproductive performance [1]. Thus, understanding the regulation of lipolysis in the dairy cow is pivotal to improve her health and productivity.

* Corresponding author. Tel.: 479-575-6872; fax: 479-575-3026.
E-mail address: delkins@uark.edu (D.A. Koltes).
† Current address: Department of Poultry Science, University of Arkansas, Fayetteville, Arkansas 72701.

http://dx.doi.org/10.1016/j.domaniend.2017.06.001

Table 1
Antibodies used for semiquantitative Western blotting.

Antibody	Catalog number[a]	Gel percentage	Dilution	Size (kDa)
PSTAT3 Try^{705}	9145	8	1:500	79,86
STAT3	8719	8	1:500	86
ATGL	2138	8	1:500	54
PHSL Ser^{563}	4139	8	1:500	81,83
PHSL Ser^{565}	4137	8	1:500	81,83
HSL	4107	8	1:500	81,83
β-actin	5125	—[b]	1:5000	45

Abbreviations: ATGL, adipose triglyceride lipase; HSL, hormone sensitive lipase; PHSL Ser^{563}, phosphorylated HSL at serine 563; PHSL Ser^{565}, phosphorylated HSL at serine 565; PSTAT3 Try^{705}, phosphorylated STAT 3 at tyrosine 705; STAT3, signal transducer and activator of transcription 3.
[a] All antibodies were purchased from Cell Signaling.
[b] β-actin antibody was exposed to all membranes.

Collectively, hormone sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) account for approximately 95% of the catecholamine-stimulated lipolytic response in mouse adipocytes [2]. Protein kinase A (PKA) is a major regulator of HSL through its phosphorylation of serine residues 563, 659, and 660 [3,4]. Phosphorylated perilipin via PKA not only allows activated HSL to access the surface of the lipid droplet [5–8] but also activates ATGL via α/β hydroxylase domain containing 5 (ABHD5) [9,10]. In addition, PKA may phosphorylate ATGL at amino acid 406 (murine) [11]. In addition to being regulated by PKA, HSL and ATGL are regulated by adenosine monophosphate–activated protein kinase (AMPK). Phosphorylation of HSL at serine 565 by AMPK is thought to induce a conformational change that precludes phosphorylation by PKA [12]. However, it is unclear if the activation of the AMPK pathway promotes [13], inhibits [12], or has no effect [14] on lipolysis. Phosphorylation of ATGL by AMPK is increased when lipolytic activity is increased in brown adipose tissue [15]. However, it should be noted that this serine residue site (ie, murine amino acid 406) may be phosphorylated by ERK [16] and may indicate a role for ERK in lipolysis.

Leptin is a 16 kDa protein secreted from adipose tissue that regulates energy homeostasis through suppression of feed intake and induction of lipolysis. Increasing intracerebral concentrations of leptin reduces feed intake in healthy gonadectomized pigs [17,18], sheep [19–22], and leptin-producing mice [23] through signaling of the janus kinase/signal transducer and activator of transcription (STAT) pathway as a result of leptin receptors activation in the hypothalamus (reviewed by [24]). In addition to acting centrally, leptin can act locally by increasing lipolysis. In vitro studies in adipocytes from rodents and pigs, leptin induces lipolysis at or above average circulating concentration of leptin via janus kinase/STAT [25–28]. Research in ruminants adipose tissue is limited to a single study in sheep that did not observe the activation of STAT3 or STAT5 [29]. Despite the potential ability of leptin to regulate adiposity, the mechanisms by which leptin induce lipolysis remain unclear. The objectives of this study were to determine (1) leptin acts directly to induce lipolysis in bovine adipocytes, (2) the potential lipases involved in leptin induced lipolysis in bovine adipocytes, and (3) increases translocation of ATGL and HSL during leptin-stimulated lipolysis in bovine stromal vascular cell–derived adipocytes.

2. Materials and methods

All procedures involving the use of animals were approved by the Iowa State University Institutional Animal Care and Use Committee (Protocol #2-11-7094-B). All cows used in this study were housed at the Iowa State University Dairy Farm located in Ames Iowa.

2.1. Materials

Dulbecco's modified eagle media (DMEM, D5523), 3-isobutyl-1-methylxanthine (IBMX, I5879), dexamethasone (D4902), insulin (I6634), and fatty acid supplement (F7175–5 mL), Free Glycerol Reagent (F6428) were purchased from Sigma Aldrich. Fetal bovine serum (FBS, s11150) was purchased from Atlanta Biologicals; antibiotic-antimycotic solution (15240) was purchased from Life Technologies; troglitazone (71750) was purchased from Caymen Chemical; and sodium acetate (S209–500g), bicinchoninic acid assay (BCA; 23227), and NEFA-HR(2) (999–34691, 995–34791, 991–34891, 993–35191, 276–76491) were purchased from Fisher Scientific. Bovine leptin (CYT-502) was purchased from ProSpec and isoproterenol (151358) was purchased from MP Biomedicals. 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI, IW-1404), bodipy (D-2184), and fluorogel with DABCO (17985–01) were purchased from IHC World, Molecular Probes, and Electron Microscopy Sciences, respectively.

2.2. Adipocyte collection

Approximately 40 g of subcutaneous adipose tissue were collected under local anesthetic (2% lidocaine) from the tailhead region of 8 lactating Holstein cows (301 ± 15 d in milk). Adipocytes were isolated using a previously described method [30–32]. Briefly, adipose tissue was transported to the laboratory in a warmed saline solution with glucose [0.15 M of sodium chloride, 1-mM HEPES, and

Table 2
Primary and secondary antibodies used for immunofluorescence.

Antibody	Company	Catalog number	Secondary antibody	Fluoroform	Catalog number[a]
PSTAT3	Cell Signaling	9145	Rabbit IgG	FITC	111-095-003
HSL	Cell Signaling	8719	Rabbit IgG	Dylight 405	711-475152
ATGL	Cell Signaling	2138	Rabbit IgG	Dylight 405	711-475-152
ABHD5	Santa Cruz Biotechnology	Sc-102285	Goat IgG	Dylight 649	705-495-003
G0S2	Novus Biologicals	4137	Mouse IgG	Dylight 594	115-515-146

Abbreviations: ABHD5, α/β hydroxylase domain containing 5; ATGL, adipose triglyceride lipase; FITC, fluorescein isothiocyanate; G0S2, G0/G1 switch protein 2; HSL, hormone sensitive lipase; PSTAT3, phosphorylated signal transducer and activator of transcription 3 at tyrosine 705.
[a] All secondary antibodies were purchased from Jackson Immunoresearch in West Grove, PA.