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Resistin induces lipolysis and suppresses adiponectin secretion in cultured human visceral adipose tissue

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ABSTRACT

Resistin is an adipokine secreted from adipose tissue, which is likely involved in the development of obesity and insulin resistance via its interaction with other organs, as well as affecting adipose tissue function. The impact of resistin treatment on lipolysis and adiponectin secretion in human visceral adipose tissue is currently unknown. Mesenteric adipose tissue samples were obtained from 14 male subjects [age 54 ± 6 yr, body mass index (BMI) 23.59 ± 0.44 kg/m^2] undergoing abdominal surgeries. Adipose tissues were cultured and treated with resistin (100 ng/mL, 24 h) in the absence or presence of different signaling inhibitors: H89 (1 μM), PD98059 (25 μM) and SB201290 (20 μM) for glycerol and non-esterified fatty acid (NEFA) measurement. Adiponectin level from media at 24 h was also measured via ELISA. Adipose tissue minces after resistin incubation (100 ng/mL, 24 h) were also collected for further Western blotting analysis.

Resistin resulted in significant induction of glycerol (3.62 ± 0.57 vs. 5.30 ± 1.11 mmol/L/g tissue, $p < 0.05$) and NEFA (5.99 ± 1.06 vs. 8.48 ± 1.57 mmol/L/g tissue, $p < 0.05$) release at 24 h. H89 and PD98059 partially inhibited resistin induced glycerol and NEFA release, while SB201290 has no such effect. Resistin induced the phosphorylation of p-HSL at serine 563, PKA at ~62 kDa and ERK1/2 as measured by Western blotting. Resistin led to significant reduction of the secretion of adiponectin (38.16 ± 10.43 vs. 21.81 ± 4.21 ng/mL/g tissue, $p < 0.05$). Our current findings implicate that resistin might play a significant role in obesity related pathologies in various tissues via its effect on adipose tissue function.

1. Introduction

It is now widely accepted that adipose tissue is an endocrine organ, secreting a host of hormone-like substances, termed as adipokines [1]. These adipose-tissue derived hormones are known to have profound physiological effects on distant tissues, such as the liver, skeletal muscle and brain [1,2]. Resistin is one of these adipokines, which was discovered by three separate laboratories [3–5]. Although mainly expressed in white adipose tissue in rodents, human resistin is expressed predominantly at macrophages, therefore it is likely that there might be species difference in regard to resistin's effects in regulating adipose tissue function. It is likely that resistin plays a role in the development of obesity and insulin resistance via its interaction with other organs [6]. Circulating resistin levels are elevated in obese and insulin resistant rodents and subjects [7,8] and are significantly down-regulated upon weight loss [9]. A causative association between resistin and systematic inflammation has also been reported [10,11]. Resistin suppresses AMP-activated protein kinase (AMPK) activation in liver and skeletal muscle [12–14]; AMPK is a well known serine/threonine kinase, as a master regulator of metabolic processes and energy homeostasis [15].

Increasing body of evidence also suggests that resistin is actively involved in affecting the function and physiology of adipose tissue per se. Resistin is up-regulated during adipocyte differentiation and is down-regulated by thiazolidinediones, a well-known PPARγ ligand [4,16]. In 3T3-L1 adipocytes, resistin increases TNF-α expression [17], and regulates plasminogen activator inhibitor-1 (PAI-1) expression [18]. Suppression of resistin expression in 3T3-L1 adipocytes via siRNA resulted in suppression of lipid production and activation of fatty acid β-oxidation, suggesting that resistin may affect lipid metabolism during adipocyte maturation [19].

Lipolysis is a unique function of adipose tissue under energy demanding conditions such as exercise and fasting [20,21]. Under

Abbreviations: AMPK, 5′ AMP-activated protein kinase; BMI, body mass index; CREB, cAMP response element binding protein; ER stress, endoplasmic reticulum stress; ERK1/2, extracellular-signal-regulated kinase 1/2; HSL, hormone sensitive lipase; NEFAs, non-esterified fatty acids; NAFLD, non-alcohol fatty liver disease; PAI-1, plasminogen activator inhibitor-1; SEM, standard error of the mean; SREBPs, sterol-regulatory-element-binding proteins; TG, triglyceride.

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these conditions, catecholamines bind to β-adrenergic receptors; consequently there is an activation of adenylyl cyclase leading to a rise in intracellular cAMP levels and the activation of PKA [22,23]. Activated PKA phosphorylates the lipid droplet protein perilipin and hormone sensitive lipase (HSL), thereby promoting hydrolysis of triglyceride (TG) [22,23]. Extracellular regulated protein kinase (ERK1/2) is another important signaling molecule involved in lipolysis [24]. It should be realized that ATGL is another important lipase involved in catecholamine stimulated lipolysis. In 3T3-L1 adipocytes, ATGL can markedly enhance both basal lipolysis and catecholamine-stimulated lipolysis [25]. ATGL-deficient mice have reduced isoproterenol stimulated lipolysis [26]. Although the endocrine regulation of lipolysis in adipose by catecholamines has been well characterized, evidence suggests that the local regulation of lipolysis in adipocytes can also be regulated by autocrine/paracrine factors. For example, adiponectin has been reported to inhibit lipolysis in human adipocytes from subcutaneous adipose tissue of non-obese individuals [27], as well as in mouse adipocytes [28]. Of primary interest to the present study, Ort et al. [29] firstly reported that in cultured human adipocytes differentiated from subcutaneous preadipocytes, human resistin can induce glycerol release in a time-dependent and dose-dependent manner, as well as in cultured mouse adipose explants and C57BL/6J mice in vivo; while mouse resistin has no effects on lipolysis in cultured primary mouse adipocytes. However, whether resistin can induce lipolysis in human visceral adipose tissue as well as the signaling pathways involved remain unclear at this timepoint. Thus, the first aim of our present study is to explore whether resistin can induce lipolytic pathways in adipose tissue.

Adiponectin is one of the most abundant adipokines secreted from adipocytes [30], which has been shown to have insulin-sensitizing activity [31] and anti-inflammatory and cardioprotective effects [32]. Adiponectin also plays a crucial role in maintaining the metabolic flexibility of adipose tissue [33]. Chronic inflammation inducing factors such as TNF-α [34], and endoplasmic reticulum stress (ER stress) [35] have been reported to suppress adiponectin expression and secretion in adipocytes. In line with this, the second aim of this study is to explore whether resistin can affect adiponectin secretion in cultured adipose tissue.

2. Materials and methods

2.1. Materials

Recombinant human resistin (cat# 450-19) was purchased from PeproTech China (NJ, USA). Human adiponectin (cat# DY1065) enzyme-linked immunosorbent assay (ELISA) kit (Duoset) was from R&D system (NE, USA). Specific inhibitors of intracellular signaling molecules SB202190 (cat# 10010399), PD98059 (cat# 10006726) and H89 (cat# 10010556) were obtained from Cayman Chemicals (KS, USA). Medium 199 (cat# 31100-035) was from Life Technologies (NY, USA). The glycerol assay kit (cat# E1002) was from Applygen Technologies (Beijing, China). The Labassay NEFA (cat# 294-63601) kit was purchased from Wako (Osaka, Japan). Reagents for SDS-PAGE were from Beyotime Institute of Technology (Jiangsu, China). Molecular weight marker and nitrocellulose membranes for SDS-PAGE were from Bio-Rad (CA, USA). Immobilon Western chemiluminescent HRP substrate (cat# WBKLS0100) was purchased from Millipore (MA, USA). Antibodies against phospho HSLser563 (cat# 4139), total HSL (cat# 4107), p-ERK1/2 (cat# 4370), ERK1/2 total (cat# 4695), phospho p38 (cat# 4511), p38 total (cat# 9212), p-PKA (cat# 9612), tubulin (cat# 2125), beta-actin (cat# 4970) and PPAR-γ (cat# 2435) were from Cell Signaling (MA, USA). Anti-ATGL antibody (cat# ab109251) was from Abcam (Cambridge, UK). Horseradish peroxidase-conjugated donkey anti-rabbit and goat anti-mouse IgG secondary antibodies were purchased from Jackson Immuno-Research Laboratories (PA, USA). Fatty acid-free bovine serum albumin (FA-free BSA) (cat# BSAS100) was from Bovogen (Melbourne, Australia). All other chemicals were purchased from Sigma (MO, USA).

2.2. Adipose tissue organ culture and treatment

The endotoxin level of recombinant human resistin (cat# 450-19) is <0.1 ng/μg of protein (i.e. <1 EU/μg). Upon arrival, human resistin was dissolved in endotoxin-free ultrapure water (Enzo Life Sciences, Inc., cat# ALX-505-008-LD15) at a stock concentration of 500 μg/mL under sterile condition and stored at −20 °C. Right before treatment, the stock resistin was diluted at a ratio of 1:100 in sterile M199 (i.e. 10 μL stock resistin dissolved in 990 μL sterile M199) and administered to the wells as designed below.

Mesenteric adipose tissue samples were obtained from 14 male subjects [age 54 ± 6 yr, body mass index (BMI) 23.59 ± 0.44 kg/m^2] undergoing abdominal surgeries of colon cancer. The samples of adipose tissue were collected during the surgery, then put in sterile PBS and immediately transported to the laboratory for experiment within 30 min. Approximately, we got 4 cases of patients for the study per week. Before bringing the patients into the study we were informed about their concomitant diseases and only patients without any acute or chronic disease except for colon cancer were included to the study. Informed consent was obtained from each donor, and this study was approved by the Human Research and Ethical Committee of the Soochow University. For the lipolysis experiment, adipose tissue (N = 7, approximately 100 mg per dish) was minced into ~5–10 mg pieces and placed into culture dishes containing 3 mL of M199 supplemented with 1% penicillin/streptomycin, 50 μU insulin and 2.5 nM dexamethasone. The cultures were placed in a cell incubator at 37 °C to equilibrate for 24 h. On the morning of the experiment, the medium was replaced with fresh M199 supplemented with 2.5% fatty acid-free BSA and treated with resistin (100 ng/mL), resistin + H89 (1 μM, an inhibitor of PKA), resistin + PD98059 (25 μM, an inhibitor of ERK1/2), and resistin + SB202190 (20 μM, an inhibitor of p38). Media were collected at 12 and 24 h for further analysis of glycerol and fatty acids. Adiponectin level from media at 24 h was also measured via ELISA. For the signaling experiment, adipose tissue (N = 7, approximately 250 mg per dish) was minced and cultured in dishes containing 7.5 mL of M199 supplemented with 1% penicillin/streptomycin, 50 μU insulin and 2.5 nM dexamethasone as described above on day 1. The next day, the medium was replaced with fresh M199 and treated with resistin (100 ng/mL) for 24 h, and adipose tissue minces were collected for further Western blotting analysis. We chose these concentrations of resistin and signal inhibitors were based on published results from other research groups [18,36,37] and preliminary data from our laboratory.

2.3. Lipolysis measurement

Culture media were analyzed for NEFA and glycerol concentrations using colorimetric assays according to the manufacturer's instructions. NEFA and glycerol concentrations were corrected for tissue weight and reported as mmol/L released per g tissue. The coefficient of variation for these assays in our laboratory is <10%.

2.4. Western blot analysis

Protein was extracted from adipose tissue and the protein content and/or phosphorylation of PKA, p38, ERK1/2 and HSL were determined by Western blotting according to Wan et al. [20] with slight modification. Briefly, adipose tissue samples were homogenized in ice-cold cell lysis buffer. Homogenized samples were centrifuged for 10 min at 12,000 *g* at 4 °C. The fat cake was removed, the infranatant was collected and the protein concentration was determined using the BCA method. Equal amounts of protein were separated on 10% gels. Proteins were wet transferred to nitrocellulose membranes at 60 mA/tank overnight