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Regulation of lipid metabolism and peroxisome proliferator-activated receptors in rainbow trout adipose tissue by lipolytic and antilipolytic endocrine factors

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ABSTRACT

The aim of this study was to determine the effects of growth hormone (GH) and insulin-like growth factor (IGF)-I on glycerol release and the regulation of IGF-I and IGF-II expression by GH in isolated rainbow trout adipocytes. Cells were also incubated with GH, tumor necrosis factor α (TNFα), or insulin to analyze the gene expression of peroxisome proliferator-activated receptors (PPARs) and lipid metabolism markers: hormone sensitive lipase, fatty acid synthase (FAS), and lipoprotein lipase. Complimentary in vivo experiments were performed by intraperitoneally administering insulin, TNFα, or lipopolysaccharide and subjecting the animals to fasting and refeeding periods. The results showed that IGF-I had an antilipolytic effect and GH had a lipolytic effect; the latter occurred independently of IGF modulation and in conjunction with a reduction in PPARα expression in adipocytes. The anabolic action of insulin was demonstrated through its upregulation of lipogenic genes such as lipoprotein lipase, FAS, and PPARγ, whereas GH, by contrast, inhibited FAS expression in adipose tissue. The gene transcription levels of PPARs changed differentially during fasting and refeeding, and the TNFα and/or lipopolysaccharide administration suggested that the regulation of PPARs helps maintain metabolic adipose tissue homeostasis in rainbow trout.

1. Introduction

Adipose tissue is a specialized organ that functions as one of the major storage sites for fat in the form of triglycerides and provides a buffer for energy imbalances. The management of fat deposition has become a key area of interest in fish farming in the quest to obtain a high-quality product with good nutritional value and to maintain fish health. The administration of high-lipid feed can lead to an unwanted increase in fat deposition that alters sensory and organoleptic characteristics [1,2]. In addition, studies have been conducted into the lipid metabolic disorders associated with the content and type of lipids in the diet [2,3] and the dietary effects on macrophage function and stress susceptibility [4,5].

In mammals, the equilibrium between lipolytic and lipogenic pathways in adipose tissue is influenced by nutritional and endocrine factors and by components of the immune response [6]. There is limited knowledge on the hormonal control of lipid turnover in fish adipose tissue [7–9]. It has been reported that growth hormone (GH), in addition to being a growth promoter, exerts a lipolytic effect in gilthead sea bream adipocytes [7,10]. GH transgenic coho salmon [11] are shown to increase their utilization of lipids for synthetic roles to maintain accelerated

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growth, but the specific effects in adipose tissue of GH overexpression remain unknown.

Insulin acts as a promoter of carbohydrate and lipid deposition by reservoir tissues during the postfeeding period in fish, although its concentration decreases during fasting periods [12]. Studies have demonstrated the anabolic role of insulin in rainbow trout adipocytes and myocytes, where it stimulates glucose and fatty acid uptake [13–16]. Insulin also stimulates lipoprotein lipase (LPL) activity in rainbow trout adipose tissue [17] and reduces the basal lipolysis level in rainbow trout and gilthead sea bream adipocytes [7,8]. Insulin-like growth factor (IGF)-I is structurally and functionally similar to insulin but is more potent as a growth factor and a metabolic controller in rainbow trout and gilthead sea bream myocytes and adipocytes [10,14,15,18]. In vertebrates, many of the growth-promoting actions of GH are known to be mediated indirectly through the stimulation of IGF transcription, mainly by the liver, or locally by extra hepatic tissues [19], but the mechanisms of action involved in GH proliferative and metabolic effects in fish are not well known [20].

In contrast, tumor necrosis factor α (TNFα) has been described as a limiting factor for adiposity in gilthead sea bream [21,22]. TNFα has been shown to promote lipolysis in isolated adipocytes of rainbow trout and gilthead sea bream [23,24]. The mechanisms underlying the action of TNFα on lipolysis are complex, and modulation of the expression of peroxisome proliferator-activated receptors (PPARs) appears to be significant [24]. There are 3 known isotypes in mammals and fish, PPARα, β, and γ, and these are expressed differentially in various tissues [25–27]. Interestingly, PPARγ is controlled by insulin in mammalian models and, during adipocyte differentiation, in rainbow trout [21]. PPARα and β have been associated with lipid catabolism such as fatty acid oxidation in the vertebrate species under study and are hormonally regulated [28–30].

There is growing evidence about the importance of fish adipose tissue in immune functions [31,32]. Bacterial lipopolysaccharide (LPS) administration is a well-known model for simulating pathogen invasion in fish [33,34]. LPS administration in fish stimulates cytokine (TNFα, IL-6, and IL-1) secretion by monocytes and macrophages and other related genes in adipose tissue [32,34] increases basal lipolysis and downregulates LPL activity in the adipocyte [23].

The aims of the present study on rainbow trout were the following: (1) to analyze the potential lipolytic action of GH in isolated adipocytes; (2) to elucidate the effects of GH, TNFα, and insulin on the expression of genes related to the lipid metabolism of adipose tissue, with a particular focus on PPARs as possible mediators of hormonal action, using in vitro and in vivo approaches; and (3) to analyze PPAR expression changes in response to a cytokine inductor (LPS) and to hormonal changes associated with fasting and refeeding.

2. Materials and methods

2.1. Animals

Rainbow trout (*Oncorhynchus mykiss* W.) were obtained from the Truites del Segre fish farm (Lleida, Spain). Fish were acclimated to a temperature of 15°C and a photoperiod of 12 h light:12 h dark in closed-circuit flow systems at the University of Barcelona's Faculty of Biology for 15 d before any experiment was conducted. They were fed daily ad libitum on a commercial diet based on fishmeal and fish oil (DibaqAquatex, Segovia, Spain).

All animal-handling procedures were approved by the Ethics and Animal Care Committee of the University of Barcelona and in accordance with regulations and procedures established by the EU and the Spanish and Catalan governments (permit reference numbers CEEA 237/12 and DAAM 6755).

2.2. Adipocyte isolation and lipolysis analysis

Animals weighing between 200 g and 250 g were killed by a blow to the head 24 h after feeding. This was followed by immersion in 70% ethanol for 30 s to sterilize the external surface. Adipocytes were isolated from mesenteric adipose tissue as described previously [7]. Adipose tissue from 4 to 5 fish was isolated and pooled together for each cell-isolation procedure to obtain adipocytes for each experiment. Tissue was cut into thin pieces and incubated in a shaking water bath at 18°C for 60 min with Krebs-Hepes buffer (pH 7.4) pregassed with 5% CO_2 in O_2 containing collagenase type II (130 U/mL) and 1% bovine serum albumin. The cell suspension was filtered through a cell strainer (100 μm) and then washed three times by flotation. Cells were carefully resuspended in Krebs-Hepes buffer containing 2% bovine serum albumin at a density of approximately 10^6 cells/mL. Cells were counted using a Fuchs-Rosenthal chamber. Aliquots of 1 mL of this final adipocyte suspension were incubated in polypropylene tubes in triplicate for 6 h at 18°C in the absence or presence of one of the following: recombinant salmon and/or trout growth hormone (GH, 0.1, 1, or 10 nM, GroPep, Berlin, Germany), recombinant human insulin (1 μM, Sigma-Aldrich, Tres Cantos, Spain) recombinant human TNF-α (rhTNFα, 100 ng/mL, Sigma-Aldrich, Tres Cantos, Spain), and recombinant human IGF-I (10 or 100 nM, Bachem, Bubendorf, Switzerland).

At the end of the period of incubation with the hormones, cells were centrifuged at 1800*g* for 2 min at 4°C. Then 300 μL of medium was placed in perchloric acid for a final concentration of 7% (vol/vol). Perchloric acid was neutralized for the measurement of glycerol concentration as an index of lipolysis using a spectrophotometric assay [35]. The remaining medium was removed, and 1 mL Tri-reagent (Ambion, Alcobendas, Spain) was added for the RNA extraction to measure IGF-I and IGF-II or fatty acid synthase (FAS), LPL, hormone sensitive lipase (HSL) and PPARα, PPARβ, and PPARγ expression (see Sections 2.5 and 2.6).

2.3. Intraperitoneal administration of insulin, TNFα, or LPS

Sixty fish were fasted for 24 h before the beginning of the experiment to avoid regurgitation of food and create metabolic basal conditions. Animals with an average weight of 176 ± 6 g and length of 22.74 ± 0.3 cm were injected intraperitoneally (1 μL/g body mass) with vehicle (phosphate buffer saline) as control, recombinant human insulin (21.6 pmol/g body mass) [36], rhTNFα (1 ng/g body mass), or LPS (*Escherichia coli*, serotype O26:B6, 6 μg/g body mass) [37], all from Sigma-Aldrich (Tres Cantos, Spain), and