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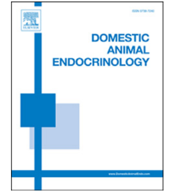

# Effects of insulin treatment on the response to oleate and octanoate of food intake and fatty acid–sensing systems in rainbow trout

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We hypothesized that food intake and the response of fatty acid (FA)-sensing systems in hypothalamus, liver, and Brockmann bodies of rainbow trout to raised levels of oleate (OL) or octanoate (OCT) is modified by insulin treatment. To assess this hypothesis, 15 fish per group received intraperitoneally 10-mL/kg injection of saline solution alone (control), or containing insulin (2-mg bovine insulin/kg body mass), OL (300 μg/kg), OCT (300 μg/kg), insulin + OL, or insulin + OCT to be sampled 6 h later to assess parameters related to FA sensing. Our results suggest that the modulatory role of insulin on the responses of hypothalamic FA-sensing systems to changes in circulating levels of OL or OCT was of minor importance in contrast to the mammalian model. However, this is in contrast with the effects observed in another experiment assessing changes in food intake after similar treatments because insulin treatment enhanced the anorectic effects of FA alone, and the effect was especially relevant ($P < 0.001$) for OCT, in contrast with the mammalian model where this FA is not inducing an anorectic response. In liver and Brockmann bodies, insulin treatment enhanced the responses to OL or OCT treatment in parameters related to FA sensing. Therefore, we provide for the first time in fish, and in a non-mammalian vertebrate, evidence for the modulation of FA-sensing systems by insulin.

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## 1. Introduction

Hypothalamic neurons in mammals respond to increases in plasma levels of long-chain fatty acid (LCFA), but not short-chain fatty acid or medium-chain fatty acid (MCFA) through several FA-sensing mechanisms [1–3]. Peripheral hormones including leptin, ghrelin, or insulin modulate this response [4]. Thus, insulin has been reported to modulate hypothalamic FA-sensitive neurons resulting in an enhancement of the FA-sensing response related to increased pro-opiomelanocortin (POMC)/cocaine- and amphetamine-related transcript (CART) expression and decreased neuropeptide Y (NPY)/agouti-related protein expression ultimately leading to decreased food intake (FI) [4–6]. These FA-sensing mechanisms in peripheral tissues such as liver and endocrine pancreas also respond to nutrients and hormones [3,7].

In fish, in previous studies in rainbow trout (*Oncorhynchus mykiss* Walbaum) [8–16], we have characterized in hypothalamus, liver, and Brockmann bodies (BB; main accumulation of pancreatic endocrine tissue in this species) the presence and functioning of putative FA-sensing systems. These systems respond to changes not only in a LCFA such as oleate (OL) but, unlike mammals, also in a MCFA-like octanoate (OCT) [16] and are related to the control of FI (hypothalamus), hormone release (BB), or metabolic homeostasis

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(liver). They are comparable to those described in mammals, and based on (1) FA metabolism through inhibition of carnitine palmitoyltransferase 1 (CPT-1) to import FA-CoA into the mitochondria for oxidation; (2) binding to FA translocase (FAT/CD36), and further modulation of transcription factors like peroxisome proliferator-activated receptor type α (PPARα), and sterol regulatory element–binding protein type 1c (SREBP1c); and (3) mitochondrial production of reactive oxygen species by electron leakage resulting in an inhibition of adenylate triphosphate (ATP)-dependent inward rectifier potassium channel ($K_{ATP}$) activity. To date, there is no evidence in fish regarding the possible endocrine modulation of these FA-sensing systems [16]. However, the cells in those FA-sensing areas are presumably the same as those involved in glucosensing [4,16,17], for which, we demonstrated in rainbow trout the existence of a modulatory role of different hormones including insulin [16,17]. Despite increased levels of circulating FA are known to elicit insulin release in fish [18], information about insulin action on lipid metabolism in fish is scarce and contradictory [19,20], and no evidence is available regarding the possible modulatory role of insulin on FA-sensing systems. In rainbow trout, the expression of insulin receptors has been demonstrated in different tissues including hypothalamus and liver [21,22]. Also, in this species, insulin administration modulates brain glucosensing system [23–25] and inhibits FI [26].

Thus, we hypothesize that FA-sensing in rainbow trout might be influenced by insulin. To assess this hypothesis, we raised OL or OCT levels in the presence of insulin and evaluated FI and the messenger RNA (mRNA) abundance in hypothalamus of neuropeptides related to the nutrient control of FI such as NPY, POMC, and CART [16]. We also evaluated in hypothalamus, liver, and BB concentrations of FA, triglyceride, total lipid, glucose, and glycogen (only in liver), as well as variables related to putative FA-sensing systems based on (1) FA metabolism, such as activities of fatty acid synthase (FAS), malonyl-CoA decarboxylase activity (MCD; only in liver), ATP-citrate lyase (ACLY; only in hypothalamus), and CPT-1, and mRNA abundance of acetyl-CoA carboxylase (ACC; only in hypothalamus), ACLY, CPT-1a (in BB), CPT-1b (in BB), CPT-1c (hypothalamus), and FAS; (2) binding to FAT/CD36 and further modulation of transcription factors, such as mRNA abundance of FAT/CD36, PPARα, SREBP1c, and liver X receptor α (LXRα); and (3) mitochondrial activity, such as mRNA abundance of mitochondrial uncoupling protein 2a (UCP2a), hydroxyacyl-CoA dehydrogenase (HOAD), inward rectifier $K^+$ channel pore type 6.x-like (Kir6.x-like), and sulfonylurea receptor-like (SUR-like; only in liver). These variables have been previously reported to change in the same species in response to increased levels of OL or OCT [8–16].

## 2. Materials and methods

### 2.1. Fish

Rainbow trout were obtained from a local fish farm (A Estrada, Spain). Fish were maintained for 1 mo before any experimental procedure in 100-L tanks under laboratory conditions and 12:12-h light-dark photoperiod in dechlorinated tap water at 15°C. Fish weight was 97 ± 3 g. Fish were fed once daily to satiety with commercial dry fish pellets (Dibaq-Diproteg SA, Spain; proximate food analysis was 48% crude protein, 14% carbohydrates, 25% crude fat, and 11.5% ash; 20.2 MJ/kg of feed). The experiments described comply with the Guidelines of the European Union Council (2010/63/UE), and of the Spanish Government (RD 55/2013) for the use of animals in research and were approved by the Ethics Committee of the Universidade de Vigo.

### 2.2. Experimental design

Fish were randomly assigned to 100-L experimental tanks. Fish were fasted for 24 h before treatment to ensure basal hormone levels were achieved.

A first set of 90 fish were randomly distributed into 6 groups of 2 tanks each (7–8 fish per tank). On the day of the experiment, fish were anesthetized with 2-phenoxyethanol (Sigma, St. Louis, MO; 0.2% v/v) and weighed. Then, 15 fish per group (fish of 2 tanks, 7–8 fish per tank) received intraperitoneally 10-mL/kg injection of saline solution alone (control), or containing insulin (I, 2-mg bovine insulin/kg body mass), OL (300 μg/kg), OCT (300 μg/kg), I + OL, or I + OCT and returned to their tanks. To safely deliver FA, they were solubilized in 45% hydroxypropyl-β-cyclodextrin (Sigma) to a final concentration of 17 mmol/L as previously described [8]. Bovine insulin has been used in many studies in rainbow trout inducing the same effects as those elicited by homologous hormone [27–29], whereas OL and OCT doses were the same as those used previously in comparable studies in vivo [8]. After 6 h of treatment, fish were anesthetized in tanks with 2-phenoxyethanol (Sigma, 0.2% v/v). Blood was collected by caudal puncture with ammonium-heparinized syringes, and plasma samples were obtained after blood centrifugation, deproteinized immediately (using 0.6-mol/L perchloric acid), and neutralized (using 1-mol/L potassium bicarbonate) before freezing and stored at −80°C until further assay. Fish were sacrificed by decapitation, and hypothalamus, liver, and BB were taken, snap-frozen, and stored at −80°C. Nine fish per group were used to assess enzyme activities and metabolite concentrations, whereas the remaining 6 fish were used for the assessment of mRNA levels by quantitative real time-polymerase chain reaction.

A second set of fish was used to evaluate changes in FI after intraperitoneal administration of FA and insulin. Sixty fish were randomly distributed into 6 tanks. FI was registered for 5 d before treatment (to define basal line data). Fish received the same intraperitoneal treatments previously described, and then FI was evaluated 6 and 24 h after treatment. After feeding, the food uneaten remaining at the bottom (conical tanks) and feed waste were withdrawn, dried, and weighed. The amount of food consumed by all fish in each tank was calculated as previously described as the difference from the feed offered [25,30,31]. The experiment was repeated 3 times; and therefore, results are shown as the mean + standard error of the mean of the data obtained in 3 different tanks per treatment.

### 2.3. Assessment of metabolite levels and enzyme activities

Concentrations of FA, triglyceride, total lipid, and glucose in plasma were determined enzymatically using commercial kits (Wako Chemicals, Neuss, Germany, for

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