Evidence for the Presence of Glucosensor Mechanisms Not Dependent on Glucokinase in Hypothalamus and Hindbrain of Rainbow Trout (*Oncorhynchus mykiss*)

Cristina Otero-Rodiño, Marta Librán-Pérez, Cristina Velasco, Marcos A. López-Patiño, Jesús M. Míguez, José L. Soengas*

Laboratorio de Fisioloxía Animal, Departamento de Bioloxia Funcional e Ciencias da Saúde, Facultade de Bioloxia, Universidade de Vigo, Vigo, Spain

* jsoengas@uvigo.es

Abstract

We hypothesize that glucosensor mechanisms other than that mediated by glucokinase (GK) operate in hypothalamus and hindbrain of the carnivorous fish species rainbow trout and stress affected them. Therefore, we evaluated in these areas changes in parameters which could be related to putative glucosensor mechanisms based on liver X receptor (LXR), mitochondrial activity, sweet taste receptor, and sodium/glucose co-transporter 1 (SGLT-1) 6h after intraperitoneal injection of 5 mL.Kg⁻¹ of saline solution alone (normoglycaemic treatment) or containing insulin (hypoglycaemic treatment, 4 mg bovine insulin.Kg⁻¹ body mass), or D-glucose (hyperglycaemic treatment, 500 mg.Kg⁻¹ body mass). Half of tanks were kept at a 10 Kg fish mass.m⁻³ and denoted as fish under normal stocking density (NSD) whereas the remaining tanks were kept at a stressful high stocking density (70 kg fish mass.m⁻³) denoted as HSD. The results obtained in non-stressed rainbow trout provide evidence, for the first time in fish, that manipulation of glucose levels induce changes in parameters which could be related to putative glucosensor systems based on LXR, mitochondrial activity and sweet taste receptor in hypothalamus, and a system based on SGLT-1 in hindbrain. Stress altered the response of parameters related to these systems to changes in glycaemia.

Introduction

Glucosensor mechanisms allow vertebrates to monitor changes in glucose levels at different central or peripheral locations [1]. Glucosensing in brain areas like hypothalamus and hindbrain has been related to the control of food intake as well as to counter-regulatory mechanisms to restore plasma levels of metabolites [1]. The mechanisms involved in glucosensing have been partially elucidated in mammals [1,2]. The most important and best characterized mechanism is that demonstrated in pancreatic β-cells and glucose-excited (GE) neurons...
dependent on glucokinase (GK), glucose facilitative transporter type 2 (GLUT2), and ATP-dependent inward rectifier potassium channel (KATP) [1]. However, since not all the glucosensing neurons rely on this mechanism [3] the existence of alternative glucosensing mechanisms has been suggested. Thus, evidence has been obtained in recent years in different peripheral and central areas of mammals, specially in omnivorous species like dog and rodents. Glucose is a direct agonist of liver X receptor (LXR) [4] whose expression is stimulated by high glucose concentrations resulting in an inhibition of gluconeogenesis [5,6]. The stimulation by glucose of sweet taste receptors (similar to those described in lingual taste cells) depending on a heterodimer of type 1 taste receptor subunits (T1Rs) formed by T1R2+T1R3 and α-gustducin, a transducin-like heterotrimeric G protein, activates an intracellular signaling cascade [7]. The expression of sodium/glucose co-transporter 1 (SGLT-1) increases in response to enhanced glucose levels in tissues like intestine [8] and brain [9,10]. Finally, another metabolism-dependent but ATP-independent mechanism suggested to contribute to hypothalamic nutrient sensing relies on mitochondrial production of reactive oxygen species (ROS) by electron leakage during intracellular glucose and fatty acid (FA) metabolism [2] leading to increased expression of uncoupling protein 2 (UCP2). In addition, several of these systems appear to be inter-connected. Thus, for instance, T1R3 and α-gustducin are necessary for increased SGLT-1 induction by dietary carbohydrates [11]. Few studies have been carried out in carnivorous mammalian species like cat regarding the presence and functioning of alternative glucosensor mechanisms, and only peripheral (but not central) areas like intestine [12] were assessed.

Glucose is not the main energy substrate of carnivorous fish species, which rely more on protein and lipid for energy purposes [13,14]. However, since glucose metabolism is important for the functioning of specific tissues like the brain [13], fish do control circulating glucose levels [1,15]. Accordingly, we have demonstrated the presence in brain areas (hypothalamus and hindbrain) of a carnivorous fish species like rainbow trout (Oncorhynchus mykiss) of a glucosensor mechanism based on GK-GLUT2-KATP similar to that characterized in mammalian GE neurons and pancreatic β-cells, which is related to the control of food intake, and to counter-regulatory mechanisms [1,13,14,15]. As for alternative glucosensor mechanisms, there is evidence obtained in fish concerning the presence of several of their components. Thus, i) orthologs of mammalian LXR are expressed in rainbow trout and Atlantic salmon tissues including brain [16,17]; ii) LXR agonists induce metabolic changes in zebrafish liver [18] and Atlantic salmon SHK-1 cells [19]; iii) UCP2 is expressed ubiquitously in gilthead sea bream [20], sea bass [21] and rainbow trout [22,23]; iv) T1Rs are present in tissues of rainbow trout [24]; and, v) SGLT-1 is present in different tissues of rainbow trout [17,24,25,26]. Moreover, in preliminary studies carried out in rainbow trout, we demonstrated the presence of components of putative glucosensor mechanisms other than that mediated by GK in peripheral tissues such as intestine [24,27] and head kidney [26]. In head kidney neither SGLT-1, Gnat3 (gene expressing α-gustducin) nor LXR mRNA abundance were affected by changes in glucose concentration [26]. In contrast, in intestine these parameters displayed changes that were dependent either in vivo or in vitro on changes in glucose levels [24]. To date, there is no available information in fish, or in any other non-mammalian vertebrate, regarding the hypothetical presence and functioning in brain areas of alternative glucosensor mechanisms. Based on our previous studies in rainbow trout, we hypothesize that alternative glucosensing mechanisms are present in hypothalamus and hindbrain responding to changes in circulating levels of glucose.

Therefore, we aimed to evaluate in hypothalamus and hindbrain of rainbow trout the presence and response to changes in glucose levels of parameters which could be related to putative alternative glucosensor mechanisms based on: i) LXR, such as fructose 1,6-bisphosphatase (FBPase) and phospho(enol)pyruvate carboxykinase (PEPCK) activities, and mRNA abundance of liver X receptor α (LXRα), FBPase, PEPCK, peroxisome proliferator-activated...
receptor type γ (PPARγ), and sterol regulatory element-binding protein type 1c (SREBP1c); ii) mitochondrial activity, such as carnitine palmitoyltransferase 1 (CPT-1) and hydroxyacil-CoA dehydrogenase (HOAD) activities, and mRNA abundance of CPT-1c, CPT-1d, HOAD, cytochrome c oxidase subunit 4 (COX4), and UCP2a; iii) sweet taste receptor, such as mRNA abundance of type 1 taste receptor subunit 2 (T1R2), type 1 taste receptor subunit 3 (T1R3), and α-gustducin (Gnat3); and, iv) SGLT-1 by assessing its mRNA abundance. As a positive control, we also evaluated changes in parameters related to GK-mediated glucosensing, such as GK and pyruvate kinase (PK) activities, and mRNA abundance of GK, GLUT2, PK, 6-phosphofructo 1-kinase (PFK), inward rectifier K+ channel pore type 6.x-like (Kir6.x-like), and sulfonyleurea receptor-like (SUR-like), as well as in the mRNA abundance of neuropeptides related to the control of food intake, such as neuropeptide Y (NPY), pro-opio melanocortin A1 (POMC-A1), cocaine and amphetamine-related transcript (CART), agouti-related peptide (AgRP), and corticotropin releasing factor (CRF).

We previously demonstrated in rainbow trout that the response of the glucosensor system based on GK is modified under stress conditions [28,29], which are also characterized by increased glycaemia, in a way that the glucosensing system is not properly informing about changes in circulating glucose levels. We therefore also aimed to evaluate if the response of putative alternative glucosensor mechanisms would be also altered under the stress conditions elicited by high stocking density.

Materials and Methods
Ethics statement
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. The Ethics Committee of the Universidade de Vigo approved the procedures.

Fish
Rainbow trout obtained from a local fish farm (A Estrada, Spain) were maintained for 1 month in 12 100L-tanks (2 replicate tanks per group containing 7–8 fish each) under laboratory conditions, 12L:12D photoperiod, and dechlorinated tap water at 15°C. Fish weight was 99 ± 3 g. Trout 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; and 20.2 MJ/kg of feed).

Experimental design
Following acclimation, fish were fasted for 24h before treatment to ensure fish had basal hormone levels. On the day of experiment, fish were anaesthetized by the addition of 2-phenox-yethanol (Sigma Chemical Co., St Louis, MO, USA; 0.2% v/v) to each tank, and weighed. Then, 15 fish per group (coming from two replicate tanks containing 7–8 fish per tank) received intraperitoneally (IP) 5 mL.Kg⁻¹ injection of saline solution alone (normoglycaemic treatment) or containing insulin (hypoglycaemic treatment, 4 mg bovine insulin.Kg⁻¹ body mass, insulin from Sigma Chemical), or D-glucose (hyperglycaemic treatment, 500 mg.Kg⁻¹ body mass). Immediately after injection, fish returned to anaesthetic-free tanks where remained for 6h without any access to food. Six tanks (two tank replicates per glycaemic treatment) were kept at 10 Kg fish mass.m⁻³ and denoted as fish under normal stocking density (NSD). In the remaining 6 tanks (two tank replicates per glycaemic treatment) a quantity of water was removed until reaching a stressful high stocking density (70 kg fish mass.m⁻³) denoted as HSD. Therefore, the
6 experimental groups used (2 tank replicates with 7–8 fish, N = 15) were maintained for 6h under different glycaemic conditions, and under different stocking densities as follows: 1) normoglycaemic fish under NSD, 2) hypoglycaemic fish under NSD, 3) hyperglycaemic fish under NSD, 4) normoglycaemic fish under HSD, 5) hypoglycaemic fish under HSD, and 6) hyperglycaemic fish under HSD. In each group, 10 fish were used to assess enzyme activities and metabolite levels whereas the remaining 5 fish were sampled for the assessment of mRNA levels by qPCR. In each sampling, fish were anesthesized as above, and blood was collected from the caudal vein with a heparinised syringe. Fish were sacrificed by decapitation, and hypothalamus and hindbrain were taken, frozen, and stored as previously described [30,31,32,33,34].

Assessment of metabolite levels and enzyme activities

Levels of glucose in plasma were determined enzymatically using a commercial kit (Biomérieux, Grenoble, France) adapted to a microplate format. Plasma cortisol levels were assessed by ELISA using a commercially available kit (Cayman, Ann Harbor, MI, USA).

Samples used to assess metabolite levels in hypothalamus and hindbrain were homogenized immediately by ultrasonic disruption in 7.5 vols of ice-cooled 0.6 M perchloric acid, and neutralized (using 1 M potassium bicarbonate). The homogenate was centrifuged (10,000 g), and the supernatant used to assay tissue metabolites. Tissue glycogen levels were assessed using the method of Keppler and Decker [35]. Glucose obtained after glycogen breakdown (after subtracting free glucose levels) was determined with a commercial kit (Biomérieux).

Samples for enzyme activities were homogenized by ultrasonic disruption in 10 vol ice-cold phosphorylation-dephosphorylation stopping buffer containing: 50 mM imidazole-HCl (pH 7.6), 15 mM 2-mercaptoethanol, 100 mM EDTA, 5 mM EGTA, and a protease inhibitor cocktail (Sigma, P-2714). The homogenate was centrifuged (10,000 g) and the supernatant used immediately for enzyme assays. Enzyme activities were determined using a microplate reader INFINITE 200 Pro (Tecan) and microplates. Reaction rates of enzymes were determined by the increase or decrease in absorbance of NAD(P)H at 340 nm or, in the case of CPT-1 activity, of 5,5'-Dithiobis(2-nitrobenzoic acid)-CoA complex at 412 nm. The reactions were started by the addition of supernatant (15 μl) at a pre-established protein concentration, omitting the substrate in control wells (final volume 265–295 μl), and allowing the reactions to proceed at 20°C for pre-established time periods. Enzyme activities are expressed per protein level, which was assayed according to the bicinchoninic acid method with bovine serum albumin (Sigma) as standard. Enzyme activities were assessed at maximum rates determined by preliminary tests to determine optimal substrate concentrations. CPT-1 (EC 2.3.1.21), FBPase (EC 3.1.3.11), GK (EC 2.7.1.2), HOAD (EC 1.1.1.35), PEPCK (EC 4.1.1.32), and PK (EC 2.7.1.40) activities were determined as described previously [30,31,32,33,34,36].

mRNA abundance analysis by real-time quantitative RT-PCR

Total RNA extracted from tissues using Trizol reagent (Life Technologies, Grand Island, NY, USA) was treated with RQ1-DNase (Promega, Madison, WI, USA). 2 μg total RNA were reverse transcribed into cDNA using Superscript II reverse transcriptase (Promega) and random hexamers (Promega). Gene expression levels were determined by real-time quantitative RT-PCR (q-PCR) using the iCycler iQ (BIO-RAD, Hercules, CA, USA). Analyses were performed using the MAXIMA SYBR Green qPCR Mastermix (Thermo Scientific, Waltham, MA, USA), in a total PCR reaction volume of 15 μl, containing 50–500 nM of each primer. mRNA abundance of transcripts (AgRP, CART, COX4, CPT-1, FBPase, GLUT2, GK, Gnat3, HOAD, Kir6.x-like, LXRα, NPY, PEPCK, PK, POMC-A1, PPARγ, SGLT-1, SREBP1c, SUR-like, T1R2, T1R3, and UCP2a) was determined as previously described in the same species
Sequences and accession numbers of the primers used for each gene expression are shown in Table 1. Relative quantification of the target gene transcripts used β-actin gene as housekeeping (stably expressed throughout the experiment).

Thermal cycling was initiated by incubation at 95°C for 90s using hot-start iTaq DNA polymerase activation; 40 steps of PCR were performed, each one consisting of heating at 95°C for 15s for denaturing, annealing at specific temperatures for 30s, and extension at 72°C for 30s. Following the final PCR cycle, melting curves were systematically monitored (temperature gradient from 55 to 95°C) to ensure amplification of only one fragment. Each sample was assessed in triplicate. Samples without reverse transcriptase and samples without RNA were run for each reaction as negative controls. Only efficiency values between 85–100% were accepted (the R$^2$ for all the genes assessed was always higher than 0.985). Relative quantification of the target gene transcript with the β-actin reference gene transcript was made following the Pfaffl method [47].

Statistics

Comparisons among groups were carried out using two-way ANOVA with glycaemic treatment (hypo-, normo-, and hyper-glycaemic) and stocking density (normal and high) as main factors. When a significant effect was noted for a factor, a Student-Newman-Keuls test was used to assess significant ($P<0.05$) differences.

Results

P-values obtained after two-way analysis of variance of results presented in the main text are shown in Table 2. P-values obtained after two-way analysis of variance of results presented in S1 Table.

Glucose levels in plasma (Fig 1A) increased in parallel with glycaemic treatment at both stocking densities with values under HSD being higher than those under NSD under normo-, and hyper-glycaemic conditions. Plasma cortisol levels (Fig 1B) displayed a significant effect of stocking density with higher levels in the HSD groups.

Glucose levels in hypothalamus (Fig 2A) increased in parallel with glycaemic treatment both under NSD and HSD conditions. Glucose levels in hindbrain (Fig 2C) increased in parallel with glycaemic treatment under NSD conditions whereas under HSD conditions levels were higher under hyper-glycaemic conditions. Glycogen levels also increased in parallel with glycaemic treatment in both areas (Fig 2B and 2D) under NSD conditions whereas under HSD conditions levels were higher in hyper-glycaemic fish in hypothalamus and lower in hypo-glycaemic fish in hindbrain.

Parameters related to glucosensing based on LXR in hypothalamus are shown in Fig 3. LXRα mRNA abundance (Fig 3A) increased in parallel with glycaemic treatment under NSD conditions whereas under HSD conditions the value of hypo-glycaemic fish was higher. FBPase activity (Fig 3B) was higher in normo-glycaemic fish than in the other glycaemic conditions for fish under NSD whereas in fish under HSD the activity in hyper-glycaemic fish was higher than that of normo-glycaemic fish. The mRNA abundance of FBPase (Fig 3C) decreased in parallel with the increase in glycaemia in NSD fish with no changes being noted under HSD. PEPCK mRNA abundance (Fig 3D) did not show changes in fish under NSD whereas in fish under HSD the value of hyper-glycaemic fish was lower than in the other two glycaemic conditions. Finally, SREBP1c mRNA abundance of fish under NSD increased in parallel with glycemia whereas in fish under HSD the value of hyper-glycaemic fish was lower than in the other two glycaemic conditions.
Parameters related to glucosensing based on LXR in hindbrain are shown in Fig 4. LXRα mRNA abundance (Fig 4) in fish under NSD was lower in hypo-glycaemic than in normo-glycaemic fish whereas in fish under HSD the value of hyperglycaemic fish was lower than in the other two glycaemic conditions. FBPase activity (Fig 4B) displayed no changes in NSD fish whereas in HSD the value of hypo-glycaemic fish was lower than those of normo-and hyperglycaemic fish. PEPCK activity (Fig 4D) in fish under NSD was lower in hyper-glycaemic than in normo-glycaemic fish whereas no changes were noted in fish under HSD. The mRNA abundance of PEPCK (Fig 4E) increased in normo-glycaemic fish compared with the two other glycaemic conditions in fish under HSD while no changes were noted for fish under NSD. The mRNA abundance of SREBP1c decreased with the increase in glycaemia in fish under HSD whereas no changes were noted in fish under NSD. Finally, no significant changes were noted for FBPase (Fig 4C) and PPARγ (Fig 4F) mRNA abundance.
Table 2. P-values obtained after two-way analysis of variance of parameters assessed in rainbow trout under different glycaemic conditions elicited by intraperitoneal (IP) administration of saline solution alone (normoglycaemic) or containing insulin (hypoglycaemic, 4 mg bovine insulin Kg\(^{-1}\) body mass), or D-glucose (hyperglycaemic, 500 mg Kg\(^{-1}\) body mass) kept at normal stocking density (NSD, 10 kg m\(^{-3}\)) or high stocking density (HSD, 70 kg m\(^{-3}\)) for 6 hours.

| Parameter     | Glycaemia | Stocking density | Glycaemia x stocking density |
|---------------|-----------|------------------|-------------------------------|
| **Plasma**    |           |                  |                               |
| Glucose levels| 0.001     | 0.001            | -                             |
| Cortisol levels|         | 0.001            | -                             |
| **Hypothalamus** |         |                  |                               |
| Glucose levels| 0.001     | -                | -                             |
| Glycogen levels| 0.034     | -                | 0.006                         |
| LXR\(\alpha\) mRNA abundance| 0.044     | -                | 0.036                         |
| FBPase activity| 0.039     | -                | -                             |
| FBPase mRNA abundance| 0.042     | -                | -                             |
| PEPCK mRNA abundance| 0.031     | -                | 0.007                         |
| PPAR\(\gamma\) mRNA abundance| 0.039     | -                | 0.034                         |
| SREBP1c mRNA abundance| 0.046     | -                | 0.003                         |
| CPT-1 activity| 0.038     | -                | 0.025                         |
| CPT1c mRNA abundance| 0.044     | -                | -                             |
| CPT1d mRNA abundance| 0.040     | -                | -                             |
| HOAD activity| 0.029     | -                | 0.045                         |
| HOAD mRNA abundance| 0.049     | -                | -                             |
| COX4 mRNA abundance| 0.004     | -                | -                             |
| UCP2a mRNA abundance| 0.011     | -                | 0.045                         |
| T1R2 mRNA abundance| 0.042     | 0.019           | -                             |
| T1R3 mRNA abundance| 0.015     | -                | 0.048                         |
| Gnat3 mRNA abundance| 0.016     | -                | -                             |
| SGLT-1 mRNA abundance| -         | -                | -                             |
| **Hindbrain** |           |                  |                               |
| Glucose levels| 0.001     | -                | 0.030                         |
| Glycogen levels| 0.001     | -                | -                             |
| LXR\(\alpha\) mRNA abundance| 0.017     | -                | -                             |
| FBPase activity| 0.047     | -                | -                             |
| FBPase mRNA abundance| -         | -                | -                             |
| PEPCK activity| 0.038     | -                | -                             |
| PEPCK mRNA abundance| 0.010     | -                | -                             |
| PPAR\(\gamma\) mRNA abundance| -         | -                | -                             |
| SREBP1c mRNA abundance| 0.005     | -                | -                             |
| CPT-1 activity| 0.046     | -                | -                             |
| CPT1c mRNA abundance| 0.012     | -                | -                             |
| CPT1d mRNA abundance| -         | -                | -                             |
| HOAD activity| -         | -                | -                             |
| HOAD mRNA abundance| 0.007     | -                | -                             |
| COX4 mRNA abundance| -         | -                | -                             |
| UCP2a mRNA abundance| 0.001     | -                | -                             |
| T1R2 mRNA abundance| 0.042     | -                | 0.034                         |
| T1R3 mRNA abundance| 0.039     | -                | -                             |
| Gnat3 mRNA abundance| -         | -                | -                             |
| SGLT-1 mRNA abundance| 0.041     | -                | 0.024                         |

Glycaemia (hypo-, normo-, and hyper-) and stocking density (NSD and HSD) were the main factors. All values are significantly different unless noted by a dash.

doi:10.1371/journal.pone.0128603.t002
The parameters associated with mitochondrial activity in hypothalamus are shown in Fig 5. CPT-1 activity (Fig 5A) increased in parallel with glycaemia in fish under NSD but no changes were noted in fish under HSD. The mRNA abundance of CPT1c (Fig 5B) in fish under NSD was higher in hyper-glycaemic than in normo-glycaemic fish whereas no changes were noted in fish under HSD. The mRNA abundance of CPT1d (Fig 5C) decreased with the increase in glycaemia in fish under HSD whereas no changes were noted for fish under NSD. HOAD activity (Fig 5D) increased in parallel with the increase in glycaemia in fish under NSD whereas in fish under HSD the value of normo-glycaemic fish was lower than in the other two glycaemic conditions. The mRNA abundance of HOAD (Fig 5E) in hyper-glycaemic fish was lower than that of normo-glycaemic fish under NSD conditions whereas in fish under HSD the value of normo-glycaemic fish was higher than in the other two glycaemic conditions. The mRNA

Fig 1. Levels of glucose (A) and cortisol (B) in plasma of rainbow trout under different glycaemic conditions elicited by intraperitoneal (IP) administration of saline solution alone (normoglycaemic) or containing insulin (hypoglycaemic, 4 mg bovine insulin.Kg⁻¹ body mass), or D-glucose (hyperglycaemic, 500 mg.Kg⁻¹ body mass) kept at normal stocking density (NSD, 10 kg.m⁻³) or high stocking density (HSD, 70 kg.m⁻³) for 6 hours. Data represent mean ± SEM of 10 measurements. *, significantly different (P<0.05) from NSD at the same glycaemic condition. Different letters indicate significant differences (P<0.05) from different glycaemic conditions at the same density (capital letters within NSD and lowercase letters within HSD).

doi:10.1371/journal.pone.0128603.g001
abundance of COX4 (Fig 5F) decreased in parallel with the increase in glycaemia both under NSD and HSD conditions. Finally, the mRNA abundance of UCP2a decreased in parallel with the increase in glycaemia in fish under HSD while no changes were noted in fish under NSD. 

The parameters associated with mitochondrial activity in hindbrain are shown in Fig 6. CPT-1 activity (Fig 6A) in normo-glycaemic fish was lower than that of hypo-glycaemic fish under HSD conditions whereas no changes were noted for fish under NSD. The mRNA abundance of CPT1c (Fig 6B) of hypo-glycaemic fish was higher than in the other two glycaemic conditions for fish under HSD while no changes occurred under NSD. The mRNA abundance of HOAD (Fig 6E) was lower in hyper-glycaemic than in normo-glycaemic fish under NSD conditions; no changes were noted under HSD. The mRNA abundance of UCP2a (Fig 6G) was higher in normo-glycaemic fish than in the other two conditions in fish under NSD whereas in fish under HSD the value was higher than that of hyper-glycaemic fish. Finally, no significant changes were noted for mRNA abundance of CPT1d (Fig 6C), HOAD activity (Fig 6D), and mRNA abundance of COX4 (Fig 6F).

The parameters related to the glucosensing mechanism based on taste receptor in hypothalamus and hindbrain are shown in Fig 7. The mRNA abundance of T1R2 in hypothalamus (Fig 7A) decreased in parallel with the increase in glycaemia in fish under HSD whereas no changes were noted in fish under NSD; the value of hypo-glycaemic fish under HSD was higher than that of fish under NSD. The mRNA abundance of T1R3 (Fig 7B) decreased in parallel with the increase in glycaemia in fish under NSD whereas in fish under HSD the value of hyper-glycaemic fish is lower than in the other two glycaemic conditions. The mRNA abundance of Gnat3 in hypothalamus (Fig 7C) decreased in parallel with the increase in glycaemia in fish under NSD whereas in
fish under HSD the value of hypo-glycaemic fish was higher than in the other two glycaemic conditions. The mRNA abundance of T1R2 in hindbrain (Fig 7D) was higher in normo-glycaemic fish than in the two other glycaemic conditions in fish under HSD whereas no changes were noted in fish under NSD. The mRNA abundance of T1R3 in hindbrain (Fig 7E) increased in hyper-glycaemic fish compared with normo-glycaemic fish under NSD whereas no changes were noted under HSD. Finally, no significant changes were noted for mRNA abundance of Gnat3 (Fig 7F).

SGLT-1 mRNA abundance was not affected by treatments in hypothalamus (Fig 8A) whereas in hindbrain (Fig 8B) values increased in parallel with glycaemia in fish under NSD while decreased under HSD.

Parameters related to glucosensing based on GK in hypothalamus are shown in S1 Fig. Significant increases in parallel with the increase in glycaemia were noted in GK activity, and

---

**Fig 3.** mRNA abundance of LXRα (A), FBPase (C), PEPCK (D), PPARγ (E), and SREBP1c (F), and activity of FBPase (B) in hypothalamus of rainbow trout. Data represent mean ± SEM of 5 (mRNA abundance) or 10 (enzyme activity) measurements. Data of mRNA abundance is expressed as fold-induction with respect to the normoglycaemic group kept under NSD (results were previously normalized by β-actin mRNA levels, which did not show changes among groups). *, significantly different (P<0.05) from NSD at the same glycaemic condition. Different letters indicate significant differences (P<0.05) from different glycaemic conditions at the same density (capital letters within NSD and lowercase letters within HSD).

doi:10.1371/journal.pone.0128603.g003
Fig 4. mRNA abundance of LXRα (A), FBPase (C), PEPCK (E), PPARγ (F), and SREBP1c (G), and activities of FBPase (B) and PEPCK (D) in hindbrain of rainbow trout. Further details as in legend of Fig 3.

doi:10.1371/journal.pone.0128603.g004
Fig 5. Activities of CPT-1 (A) and HOAD (D), and mRNA abundance of CPT1c (B), CPT1d (C), HOAD (E), COX4 (F), and UCP2a (G) in hypothalamus of rainbow trout. Further details as in legend of Fig 3.

doi:10.1371/journal.pone.0128603.g005
Fig 6. Activities of CPT-1 (A) and HOAD (D), and mRNA abundance of CPT1c (B), CPT1d (C), HOAD (E), COX4 (F), and UCP2a (G) in hindbrain of rainbow trout. Further details as in legend of Fig 3.

doi:10.1371/journal.pone.0128603.g006
mRNA abundance of GK and PK whereas a significant decrease was noted for GLUT2 mRNA abundance, and significant changes were also noted for Kir6.x-like mRNA abundance. Stocking density significantly decreased GK activity whereas significant interactions between glycaemic treatment and stocking density were noted for all parameters (except mRNA abundance of GLUT2 and Kir6.x-like).

Parameters related to glucosensing based on GK in hindbrain are shown in S2 Fig. Significant increases in parallel with the increase in glycaemia were noted in GK and PK activities, and mRNA abundance of GK, PK, and PFK whereas significant changes were also noted for Kir6.x-like mRNA abundance. Stocking density significantly affected mRNA abundance of GLUT2 and PK whereas only for GK activity a significant interaction between stocking density and glycaemic treatment was noted.

Fig 7. mRNA abundance of T1R2 (A,D), T1R3 (B,E), and Gnat3 (C,F) in hypothalamus (A,B,C) and hindbrain (D,E,F) of rainbow trout. Further details as in legend of Fig 3.

doi:10.1371/journal.pone.0128603.g007
Finally, mRNA abundance of neuropeptides is shown in S3 Fig. All parameters assessed except AgRP and NPY were affected by treatments. Glycaemic treatment increased mRNA abundance of POMC-A1 and CART in hypothalamus in hyperglycaemic fish whereas levels of CART were higher in normoglycaemic fish in hindbrain. Significant interactions between glycaemic treatment and stocking density were observed in mRNA abundance of POMC-A1 and CRF in hypothalamus and CART in hindbrain.

Discussion

Validation of experimental design

The experimental design aimed to induce changes in circulating levels of glucose similar to those previously observed in the same species, which were able to induce a response in the gluco-sensing system based on GK-GLUT2-K\textsubscript{ATP} [1,13,14]. Plasma levels of glucose decreased after hypo-glycaemic treatment and increased after hyper-glycaemic treatment. Similar changes occurred in glucose levels in hypothalamus and hindbrain. The different glycaemic conditions did not alter plasma cortisol levels in non-stressed fish whereas in stressed fish levels were higher than those of non-stressed fish at any glycaemic conditions. We have not used uninjected fish but levels of plasma glucose and cortisol in normo-glycaemic fish under NSD are similar to those observed in uninjected fish in previous studies thus suggesting a minor (if any) stress effect of injection. The parameters related to the glucosensor system based on GK in hypothalamus and hindbrain of fish under NSD conditions displayed changes in parallel with the increase in glycaemia (see supplementary information). These include GK activity and mRNA abundance, glycogen levels, glycolytic capacity (PK activity, and mRNA abundance of PK and PFK) and mRNA abundance of GLUT2 and components of the K\textsubscript{ATP} channel. In general, these results, together with those of mRNA abundance of neuropeptides related to the control of food intake, are in agreement with those previously reported under similar experimental conditions in the same species [29,30,31,48] thus validating the experimental design.

In the next paragraphs we will discuss whether or not parameters which could be related to putative alternative gluco-sensing systems respond to the changes elicited in glycaemia. Since hypo-glycaemia was obtained through insulin treatment, we cannot discard that at least part of the effects observed under hypo-glycaemic conditions in all parameters assessed could be attributed to a direct insulin action.
Glucosensing mediated by LXR

A glucosensor system based on the nuclear receptor LXR has been described in mammalian liver [5]. An increase in glucose levels activates this receptor resulting in decreased gluconeogenic capacity [6], and increased mRNA abundance of SREBP1c [49] and PPARγ [50,51]. We demonstrated in rainbow trout that LXRα mRNA abundance in intestine increased in response to elevated glucose levels either in vivo or in vitro [24] whereas in head kidney no response was noted when glucose levels were modified [26]. In the present study, a putative LXR-mediated glucosensor system could be operative in hypothalamus since increased LXRα mRNA abundance occurred in parallel with the increase in glycaemia. We have also assessed several parameters related to gluconeogenesis observing a decrease in the abundance of FBPase mRNA in parallel with the increase in glycaemia whereas a significant decrease in FBPase activity was observed when comparing normo- vs. hyper-glycaemic fish. These changes suggest an effective inhibition of gluconeogenic capacity in response to increased glucose in a way similar to that reported in mammalian liver [4,52] and to the decreased PEPCK mRNA abundance reported in zebrafish exposed to glucose [53]. Furthermore, we also observed clear changes in the mRNA abundance of transcription factors related to LXR activity such as SREBP1c and PPARγ [50,51] that displayed changes in parallel with those of glucose levels giving further support to the presence and functioning of a LXR-based glucosensing system in rainbow trout hypothalamus. The increase noted in SREBP1c mRNA abundance in response to increase glucose levels is similar to that characterized in mammalian liver [6], and in rainbow trout fed a diet rich in carbohydrates [23] and could be related to the activation of LXR as demonstrated previously in Atlantic salmon liver [19].

In contrast, in hindbrain the few changes observed in response to increased levels of glucose were not in agreement with those expected for a glucosensor system based on LXR since for instance FBPase activity increased, instead of the expected decrease resulting from decreased gluconeogenic potential.

Glucosensing mediated by mitochondrial activity

Another metabolism-dependent but ATP-independent mechanism suggested to contribute to hypothalamic sensing of glucose and fatty acid in mammals relies on enhanced mitochondrial production of ROS by electron leakage, which is buffered by UCP2a [2,54] in a way that glucose sensing is negatively regulated by raised UCP2 activity [55,56].

In the present study, we observed in hypothalamus, but not in hindbrain, a clear increase in the activity of CPT-1 and HOAD in parallel with the increased glucose. The increased activity of both enzymes is clearly suggesting an enhanced oxidative capacity in the mitochondria in response to enhanced glucose levels. These changes are also comparable with increased mRNA abundance of CPT-1 observed in liver of rainbow trout fed with a diet rich in carbohydrates [23]. Moreover, we also observed changes in the mRNA abundance of parameters which could be related to mitochondrial activity, and these include a clear decrease in the mRNA abundance of COX4 in parallel with the increase in glucose as well as a decrease in HOAD mRNA abundance, and an increase in CPT1c when comparing hyperglycaemic vs. normo-glycaemic fish. These changes suggest the existence of enhanced mitochondrial activity in response to increased glucose, which based on the mammalian model would presumably result in enhanced ROS production. However, we did not observe the expected increase in UCP2a mRNA abundance. This is comparable to the lack of changes also noted in UCP2a mRNA abundance in hypothalamus of another fish species (orange-spotted grouper) under postprandial conditions [57] when a rise in metabolite levels (including glucose) is expected, as well as with the lack of changes observed in rainbow trout hepatocytes exposed to glucose [17]. As a whole, these results provide only
partial support to the presence of a putative glucosensor system based on mitochondrial activity in trout hypothalamus. However, we cannot discard the possibility that changes in mitochondrial activity may be also dependent on the GK glucose sensing mechanism.

**Glucosensing mediated by sweet taste receptor**

The transduction of the sweet taste relates to the detection of sugars including glucose in a heterodimer complex formed by T1R2+T1R3 and α-gustducin. This complex is present not only in lingual areas in mammals but also in other areas such as intestine [58], pancreatic β-cells [59] or hypothalamus [7] where is involved in glucosensing. In fish, we previously demonstrated in rainbow trout the presence of components of this system in both intestine [24] and head kidney [26]. However, only in intestine [24] a response of the evaluated parameters to changes in glucose levels was evident.

In the present study, we observed in hypothalamus, in parallel with the increase in glucose levels, a clear decrease in the mRNA abundance of T1R3 and Gnat3. This change is comparable to that already demonstrated in mammalian pancreatic β-cells [60] under similar conditions as well to that also observed in rainbow trout intestine [24]. However, rat hypothalamus responds to increased glucose levels in vitro with decreased T1R2 mRNA abundance but without changes in T1R3 and Gnat3 mRNA abundance [7]. In contrast, in rainbow trout, we observed decreased levels of T1R3 and Gnat3 mRNA abundance in both hypothalamus (this study) and intestine [24]. Therefore, we may suggest the existence of a different model of response of transcript abundance when comparing fish and mammals concerning glucosensing based on sweet taste receptor. In contrast, in hindbrain these changes were not observed and even an increase under hyper-glycaemic conditions was observed in that brain area.

**Glucosensing mediated by SGLT-1**

SGLT-1 may act as a glucosensor by conveying information to the cell about external glucose concentration, directly through the membrane potential or indirectly coupled through a G protein [9]. Thus, the increase in glucose levels induce increased expression of SGLT-1 in different tissues of mammals thus functioning as an effective glucosensor [61]. In fish, there is evidence for the presence of this carrier in different tissues [24,25,26,62] but only in intestine there was evidence for its possible role a as glucosensor since its expression enhanced in response to increased levels of glucose [24]. In the present study, we did not observe any change in mRNA abundance of SGLT-1 in hypothalamus whereas a clear increase in hindbrain occurred in parallel with the increase in glucose. Therefore, these results suggest that SGLT-1 in hindbrain is modulated by glucose levels.

**Stress induced by high stocking density modifies the response of glucosensing parameters**

Stress induced by HSD resulted in increased levels of cortisol and glucose without inducing any significant change in normo-glycaemic fish. A similar lack of effects of stress on parameters related to glucosensing based on GK was previously observed in rainbow trout under similar experimental conditions [29]. In hypothalamus, the glucosensor system based on GK did not respond to changes in glucose levels in stressed fish (see supplementary information). These results are similar to those previously reported in the same tissue and species [28,29] and are in agreement with the results observed in the expression of neuropeptides.

In the parameters which could be related to putative glucosensor system based on LXR, mitochondrial activity and sweet taste receptor in hypothalamus, and SGLT-1 in hindbrain, an interaction between glycaemic conditions and stress induced by HSD was noted in several
parameters. Thus, the response of these parameters to changes in glucose levels is altered under the stress conditions elicited by high stocking density when compared with non-stressed fish. The precise connection between stress and the function of alternative glucosensing mechanisms has to be established in further studies. However, the effects of stress on the systems could relate to the action of any of the components of the hypothalamus-pituitary-interrenal (HPI) axis, such as CRF whose mRNA abundance displayed changes in hypothalamus of stressed fish depending on glycaemia, as demonstrated previously in the modulation by CRF of the glucosensor system based on GK in rainbow trout hypothalamus [63].

Conclusions

The results obtained in non-stressed rainbow trout evidence for the first time in fish, and in a non-mammalian vertebrate, that manipulation of glucose levels affects parameters which could be related to putative glucosensor systems based on LXR, mitochondrial activity, and sweet taste receptor in hypothalamus and on SGLT-1 in hindbrain. We cannot discard, however, that some of the changes may be also due to GK activation. This differential response between brain areas is different than that of GK-mediated system, which responded to glucose similarly in both brain areas [1,13,14], and to that known in the mammalian model [1]. Despite rainbow trout is a carnivorous species, the responses observed for alternative glucosensing systems in hypothalamus are more comparable in general to those described in omnivorous mammals [3] in the same area than to those observed in peripheral areas, like intestine, in the few available studies in carnivorous mammals like cats [12]. The stress conditions elicited by HSD altered the response to glucose of parameters that could be associated with putative glucosensor systems in hypothalamus and hindbrain. Further studies are necessary to characterize the underlying mechanisms and the physiological role (control of food intake, counter-regulatory mechanisms) of these putative alternative glucosensing capabilities of rainbow trout brain.

Supporting Information

S1 Table. P-values obtained after two-way analysis of variance. Parameters related to GK-mediated glucosensing were assessed in rainbow trout under different glycaemic conditions elicited by intraperitoneal (IP) administration of saline solution alone (normoglycaemic) or containing insulin (hypoglycaemic, 4 mg bovine insulin.Kg⁻¹ body mass), or D-glucose (hyperglycaemic, 500 mg.Kg⁻¹ body mass) kept at normal stocking density (NSD, 10 kg.m⁻³) or high stocking density (HSD, 70 kg.m⁻³) for 6 hours. Glycaemia (hypo-, normo-, and hyper-) and stocking density (NSD and HSD) were the main factors. All values are significantly different unless noted by a dash.

S1 Fig. Parameters related to glucosensing based on GK in hypothalamus. mRNA abundance of GLUT2 (A), GK (C), PK (D), PFK (E), Kir6.x-like (F) and SUR-like (G) and activity of GK (B) in hypothalamus of rainbow trout. Data represent mean ± SEM of 5 (mRNA abundance) or 10 (enzyme activity) measurements. Data of mRNA abundance is expressed as fold-induction with respect to the normoglycaemic group kept under NSD (results were previously normalized by β-actin mRNA levels, which did not show changes among groups). * significantly different (P<0.05) from NSD at the same glycaemic condition. Different letters indicate significant differences (P<0.05) from different glycaemic conditions at the same density (capital letters within NSD and lowercase letters within HSD).
S2 Fig. Parameters related to glucosensing based on GK in hindbrain. mRNA abundance of GLUT2 (A), GK (C), PK (E), PFK (F), Kir6.x-like (G) and SUR-like (H) and activities of GK (B) and PK (D) in hindbrain of rainbow trout. Data represent mean ± SEM of 5 (mRNA abundance) or 10 (enzyme activity) measurements. Data of mRNA abundance is expressed as fold-induction with respect to the normoglycaemic group kept under NSD (results were previously normalized by β-actin mRNA levels, which did not show changes among groups). *, significantly different (P<0.05) from NSD at the same glycaemic condition. Different letters indicate significant differences (P<0.05) from different glycaemic conditions at the same density (capital letters within NSD and lowercase letters within HSD).

(TIF)

S3 Fig. Neuropeptide expression. mRNA abundance of AgRP (A,F), NPY (B,G), POMC-A1 (C,H), CART (D,I), and CRF (E,J) in hypothalamus (A,B,C,D,E) and hindbrain (F,G,H,I,J) of rainbow trout. Data represent mean ± SEM of 5 measurements. Data of mRNA abundance is expressed as fold-induction with respect to the normoglycaemic group kept under NSD (results were previously normalized by β-actin mRNA levels, which did not show changes among groups). *, significantly different (P<0.05) from NSD at the same glycaemic condition. Different letters indicate significant differences (P<0.05) from different glycaemic conditions at the same density (capital letters within NSD and lowercase letters within HSD).

(TIF)

Author Contributions
Conceived and designed the experiments: COR MALP JMM JLS. Performed the experiments: COR MLP CV. Analyzed the data: COR MLP CV MALP. Contributed reagents/materials/analysis tools: JMM JLS. Wrote the paper: COR MLP CV MALP JMM JLS.

References
1. Polakof S, Mommsen TP, Soengas JL. Glucosensing and glucose homeostasis: from fish to mammals. Comp Biochem Physiol B. 2011; 160: 123–149. doi:10.1016/j.cbpb.2011.07.006 PMID: 21871969
2. Blouet C, Schwartz GJ. Hypothalamic nutrient sensing in the control of energy homeostasis. Behav. Brain Res. 2010; 209: 1–12. doi:10.1016/j.bbr.2009.12.024 PMID: 20035790
3. Fioramonti X, Lorsignol A, Taupignon A, Pénicaud L. A new ATP-sensitive K+ channel-independent mechanism is involved in glucose-excited neurons of mouse arcuate nucleus. Diabetes. 2004; 53: 2767–2775. PMID:15504956
4. Mitro N, Mak PA, Vargas L, Godio C, Hampton E, Molteni V, et al. The nuclear receptor LXR is a glucose sensor. Nature, 2007; 445: 219–223. PMID: 17187055
5. Anthonisen EH., Berven L, Holm S, Nygård M, Nebb HI, Grønning-Wang LM. Nuclear receptor liver X receptor is O-GlcNAc-modified in response to glucose. J Biol Chem. 2010; 285: 1607–1615. doi: 10.1074/jbc.M109.082685 PMID: 19933273
6. Archer A, Laurencikiene J, Ahmed O, Steffensen KR, Parini P, Gustafsson J- A. et al. Skeletal muscle as a target of LXR agonist after long-term treatment: focus on lipid homeostasis. Am J Physiol Endocrinol Metab. 2014; 306: E494–E502. doi: 10.1152/ajpendo.00410.2013 PMID: 24368671
7. Ren X, Zhou L, Terwilliger R, Newton SS, de Araujo IE. Sweet taste signaling functions as a hypothalamic glucose sensor. Front Integr Neurosci. 2009; 3: 1–15. doi: 10.3389/neuro.07.001.2009 PMID: 19225578
8. Margolskee RF, Dyer J, Kokrashvili Z, Salmon KSH, Ilegems E, Daily K, et al. T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1. Proc Natl Acad Sci USA 2007; 104: 15075–15080. PMID: 17724332
9. Diez-Sampedro A, Hirayama BA, Osswald C, Gorbulev V, Baumgarten K, Volk C, et al. A glucose sensor hiding in a family of transporters. Proc Natl Acad Sci USA. 2003; 100: 11753–11758. PMID: 13130073
10. González JA, Reimann F, Burdakov D. Dissociation between sensing and metabolism of glucose in sugar sensing neurones. J Physiol. 2009; 587: 41–48. doi: 10.1113/jphysiol.2008.163410 PMID: 18981030

11. Wauson EM, Lorente-Rodríguez A, Cobb MH. Minireview: nutrient sensing by G protein-couples receptors. Mol Endocrinol. 2013; 27: 1188–1197. doi: 10.1210/me.2013-1100 PMID: 23820899

12. Batchelor DJ, Al-Rammahi M, Moran AW, Brand JG, Li X, Haskins M, et al. Sodium/glucose cotransporter-1, sweet receptor, and disaccharidase expression in the intestine of the domestic dog and cat: two species of different dietary habit. Am J Physiol Regul Integr Comp Physiol. 2011; 300: R67–R75. doi: 10.1152/ajpregu.00262.2010 PMID: 20980625

13. Polakof S, Panserat S, Soengas JL, Moon TW. Glucose metabolism in fish: a review. J Comp Physiol B. 2012; 182: 1015–1045. doi: 10.1007/s00360-012-0658-7 PMID: 22476584

14. Soengas JL. Contribution of glucose- and fatty acid sensing systems to the regulation of food intake in fish. A review. Gen Comp Endocrinol. 2014; 205: 36–48. doi: 10.1016/j.ygcen.2014.01.015 PMID: 24530522

15. Soengas JL, Polakof S (2013) Glucosensing in rainbow trout. In: Polakof S, Moon TW, editors. Trout: from physiology to conservation; 2013. pp. 155–177.

16. Cruz-Garcia L, Sánchez-Gurmaches J, Gutiérrez J, Navarro I. Role of LXR in trout adipocytes: target genes, hormonal regulation, adipocyte differentiation and relation to lipolysis. Comp Biochem Physiol A. 2012; 163: 120–126. doi: 10.1016/j.cbpa.2012.05.193 PMID: 22626869

17. Craig PM, Massarsky A, Moon TW. Understanding glucose uptake during methionine deprivation in incubated rainbow trout (Oncorhynchus mykiss) hepatocytes using a non-radioactive method. Comp Biochem Physiol B. 2013; 166: 23–29. doi: 10.1016/j.cbpb.2013.06.005 PMID: 23796851

18. Sukardi H, Zhang X, Lui EY, Ung CY, Mathavan S, Gong Z, et al. Liver X receptor agonist T0901317 induces liver perturbation in zebrafish: histological, gene set enrichment and expression analysis. Biochim Biophys Acta. 2012; 1820: 33–43. doi: 10.1016/j.bbagen.2011.10.009 PMID: 22047996

19. Carmona-Antofañanzas G, Tocher DR, Martinez-Rubio L, Leaver MJ. Conservation of lipid metabolic gene transcriptional regulatory networks in fish and mammals. Gene. 2014; 534: 1–9. doi: 10.1016/j.gene.2013.10.040 PMID: 24177230

20. Bermejo-Nogales A, Calduch-Giner JA, Pérez-Sánchez J. Gene expression survey of mitochondrial uncoupling proteins (UCP1/UCP3) in gilthead sea bream (Sparus aurata L.). J Comp Physiol B. 2010; 180: 685–694. doi: 10.1007/s00360-009-0441-6 PMID: 20063001

21. Tine M, Kuhl H, Jastroch M, Reinhardt R. Genomic characterization of the European sea bass (Dicentrarchus labrax) reveals the presence of a novel uncoupling protein (UCP) gene family member in the teleost fish lineage. BMC Evol Biol. 2012; 12: 62. doi: 10.1186/1471-2148-12-62 PMID: 22577775

22. Coulibaly I, Gahr SA, Palt Y, Yao J, Rexroad CE III. Genomic structure and expression of uncoupling protein 2 genes in rainbow trout (Oncorhynchus mykiss). BMC Genomics. 2006; 7: 203. PMID: 16899121

23. Craig PM, Moon TW. Methionine restriction affects the phenotypic and transcriptional response of rainbow trout (Oncorhynchus mykiss) to carbohydrate-enriched diets. Brit J Nutr. 2013; 109: 402–412. doi: 10.1017/S0007114512001663 PMID: 22583536

24. Polakof S, Soengas JL. Evidence of sugar sensitive genes in the gut of a carnivorous fish species. Comp Biochem Physiol B. 2013; 166: 58–64. doi: 10.1016/j.cbpb.2013.07.003 PMID: 23850750

25. Sugiuira SH, McDaniel NK, Ferraris RP. In vivo fractional Pi absorption and NaPi-II mRNA expression in rainbow trout are upregulated by dietary P restriction. Am J Physiol Regul Integr Comp Physiol. 2003; 285: R770–R781. PMID: 12816744

26. Conde-Sieira M, Álvarez R, López-Patiño MA, Míquez JM, Flik G, Soengas JL. ACTH-stimulated cortisol release from head kidney of rainbow trout is modulated by glucose concentration. J Exp Biol. 2013; 216: 554–567. doi: 10.1242/jeb.076505 PMID: 23077165

27. Polakof S, Álvarez R, Soengas JL. Gut glucose metabolism in rainbow trout: implications in glucose homeostasis and glucosensing capacity. Am J Physiol Regul Integr Comp Physiol. 2010; 299: R19–R32. doi: 10.1152/ajpregu.00005.2010 PMID: 20357022

28. Conde-Sieira M, Aguilar AJ, López-Patiño MA, Míquez JM, Soengas JL. Stress alters food intake and glucosensing response in hypothalamus, hindbrain, liver, and Brockenmann bodies of rainbow trout. Physiol Behav. 2010; 101: 483–493. doi: 10.1016/j.physbeh.2010.07.016 PMID: 20688089

29. Conde-Sieira M, Aguileño MJ, Aguilar AJ, Míquez JM, Cerdá-Reverter JM, Soengas JL. Effect of different glycaemic conditions on gene expression of neuropeptides involved in control of food intake in rainbow trout; interaction with stress. J Exp Biol. 2010; 213: 3858–3865. doi: 10.1242/jeb.048439 PMID: 21037065
30. Polakof S, Míguez JM, Moon TW, Soengas JL. Evidence for the presence of a glucosensor in hypothalamus, hindbrain, and Brockmann bodies of rainbow trout. Am J Physiol Regul Integr Comp Physiol. 2007; 292: R1657–R1666. PMID: 17170235

31. Polakof S, Míguez JM, Soengas JL. In vitro evidences for glucosensing capacity and mechanisms in hypothalamus, hindbrain, and Brockmann bodies of rainbow trout. Am J Physiol Regul Integr Comp Physiol. 2007; 293: R1410–R1420. PMID: 17567722

32. Polakof S, Míguez JM, Soengas JL. Changes in food intake and glucosensing function of hypothalamus and hindbrain in rainbow trout subjected to hyperglycemic or hypoglycemic conditions. J Comp Physiol A. 2008; 194: 829–839. doi: 10.1007/s00359-008-0354-y PMID: 18663455

33. Polakof S, Míguez JM, Soengas JL. Dietary carbohydrates induce changes in glucosensing capacity and food intake in rainbow trout. Am J Physiol Regul Integr Comp Physiol. 2008; 295: R478–R489. doi: 10.1152/ajpregu.00176.2008 PMID: 18525014

34. Polakof S, Panserat S, Plagnes-Juan E, Soengas JL. Altered dietary carbohydrates significantly affect gene expression of the major glucosensing components in Brockmann bodies and hypothalamus of rainbow trout. Am J Physiol Regul Integr Comp Physiol. 2008; 295: R1077–R1088. doi: 10.1152/ajpregu.90476.2008 PMID: 18685066

35. Keppeler D, Decker K. Glycogen determination with amyloglucosidase. In: Bergmeyer HU, editor. Methods of Enzymatic Analysis; 1974. pp. 1127–1131.

36. Librán-Pérez M, López-Patho MA, Míguez JM, Soengas JL. Oleic acid and octanoic acid sensing capacity in rainbow trout Oncorhynchus mykiss is direct in hypothalamus and Brockmann bodies. PLoS ONE. 2013; 8: e59507. doi: 10.1371/journal.pone.0059507 PMID: 23533628

37. Panserat S, Médale F, Bérénché J, Vachot C, Plagnes-Juan E, et al. Haptic glucokinase is induced by dietary carbohydrates in rainbow trout, gilthead seabream, and common carp. Am J Physiol Regul Integr Comp Physiol. 2000; 278: R1164–R1170. PMID: 10801283

38. Panserat S, Plagnes-Juan E, Kaushik S. Nutritional regulation and tissue specificity of gene expression for proteins involved in hepatic glucose metabolism in rainbow trout (Oncorhynchus mykiss), J Exp Biol. 2001; 204: 2351–2360. PMID: 11507117

39. Polakof S, Médale F, Skiba-Cassy S, Corraze G, Panserat S. Molecular regulation of lipid metabolism in liver and muscle of rainbow trout subjected to acute and chronic insulin treatments. Domestic Anim Endocrinol. 2010; 39: 26–33. doi: 10.1016/j.domaniend.2010.01.003 PMID: 20181454

40. Polakof S, Médale F, Larroquet L, Vachot C, Corraze G, Panserat S. Insulin stimulates lipogenesis and attenuates beta-oxidation in white adipose tissue of fed rainbow trout. Lipids. 2011; 46: 189–199. doi: 10.1007/s11745-010-3521-1 PMID: 21240564

41. Leder EH, Silverstein JT. The pro-opiomelanocortin genes in rainbow trout (Oncorhynchus mykiss): duplications, splice variants, and differential expression. J Endocrinol. 2006; 188: 355–363. PMID: 16461561

42. Bernier NJ, Alderman SL, Bristow EN. Heads or tails? Stressor-specific expression of corticotropin-releasing factor and urotensin I in the preoptic area and caudal neurosecretory system of rainbow trout. J Endocrinol. 2008; 196: 637–648. doi: 10.1677/JOE-07-0568 PMID: 18310459

43. Kolditz C, Bornhaire M, Richard N, Corraze G, Panserat S. Insulin stimulates lipogenesis and attenuates beta-oxidation in white adipose tissue of fed rainbow trout. Lipids. 2011; 46: 189–199. doi: 10.1007/s11745-010-3521-1 PMID: 21240564

44. Kolditz C, Bornhaire M, Richard N, Corraze G, Panserat S, Vachot C, et al. Liver and muscle metabolic changes induced by dietary energy content and genetic selection in rainbow trout (Oncorhynchus mykiss). Am J Physiol Regul Integr Comp Physiol. 2008; 294: R1154–R1164. doi: 10.1152/ajpregu.00766.2007 PMID: 18234747

45. Lansard M, Panserat S, Seillez I, Polakof S, Plagnes-Juan E, Geurden I, et al. Hepatic protein kinase B (Akt)-target of rapamycin (TOR)-signalling pathways and intermediary metabolism in rainbow trout (Oncorhynchus mykiss) are not significantly affected by feeding plant-based diets. Brit J Nutr. 2009; 102: 1564–1573. doi: 10.1017/S000711450999095X PMID: 19664314

46. Cruz-Garcia L, Minghetti M, Navarro I, Tocher DR. Molecular cloning, tissue expression and regulation of liver X receptor (LXR) transcription factors of Atlantic salmon (Salmo salar) and rainbow trout (Oncorhynchus mykiss). Comp Biochem Physiol B. 2009; 153: 81–88. doi: 10.1016/j.cbpb.2009.02.001 PMID: 19416695

47. Sánchez-Gurmaches J, Cruz-Garcia L, Gutiérrez J, Navarro I. Adiponectin effects and gene expression in rainbow trout: an in vivo and in vitro approach. J Exp Biol. 2012; 215: 1373–1383. doi: 10.1242/jeb.069740 PMID: 22660781

48. Conde-Sieira M, López-Patho MA, Míguez JM, Soengas JL. Glucosensing capacity in rainbow trout liver displays day-night variations possibly related to melatonin action. J Exp Biol. 2012; 215: 3112–3119. doi: 10.1242/jeb.069740 PMID: 22660781
49. Higuchi N, Kato M, Miyazaki M, Tanaka M, Kohjima M, Ito T, et al. Potential role of branched-chain amino acids in glucose metabolism through the accelerated induction of the glucose-sensing apparatus in the liver. J Cell Biochem. 2011; 112: 30–38. doi: 10.1002/jcb.22688 PMID: 20506195

50. Kim H-I, Ahn Y-H. Role of peroxisome proliferator-activated receptor-γ in the glucose-sensing apparatus of liver and β-cells. Diabetes. 2004; 53 (Suppl 1): S60–S65. PMID: 14749267

51. Festuccia WT, Blanchard P-G, Belchior T, Chimin P, Paschoal VA, Magdaloni J, et al. PPARγ activation attenuates glucose intolerance induced by mTOR inhibition with rapamycin in rats. Am J Physiol Endocrinol Metab. 2014; 306: E1046–E1054. doi: 10.1152/ajpendo.00683.2013 PMID: 24619883

52. Steffensen KR, Gustafsson J-A. Putative metabolic effects of the liver X receptor (LXR). Diabetes. 2004; 53 (Suppl. 1): S42.

53. Elo B, Villano CM, Govorko D, White LA. Larval zebrafish as a model for glucose metabolism: expression of phosphoenolpyruvate carboxykinase as a marker for exposure to anti-diabetic compounds. J Mol Endocrinol. 2007; 38: 433–440. PMID: 17446233

54. De Morentin PBM, González CR, Saha AK, Martins L, Diéguez C, Vidal-Puig A, et al. PPARγ activation attenuates glucose intolerance induced by mTOR inhibition with rapamycin in rats. Am J Physiol Endocrinol Metab. 2014; 306: E1046–E1054. doi: 10.1152/ajpendo.00683.2013 PMID: 24619883

55. Kong D, Vong L, Parton LE, Ye C, Tong Q, Hu X, et al. Glucose stimulation of hypothalamic MCH neurons involves KATP channels, is modulated by UCP2, and regulates peripheral glucose homeostasis. Cell Metab. 2010; 12: 545–552. doi: 10.1016/j.cmet.2010.09.013 PMID: 21035764

56. Beall C, Hamilton DL, Gallagher J, Logie L, Wright K, Soutar MP, et al. Mouse hypothalamic GT1-7 cells demonstrate AMPK-dependent intrinsic glucose-sensing behaviour. Diabetologia. 2012; 55: 2432–2444. doi: 10.1007/s00125-012-2617-y PMID: 22760787

57. Tang Z, Sun C, Yan A, Wu S, Qin C, Zhang Y, et al. Genes involved in fatty acid metabolism: molecular characterization and hypothalamic mRNA response to energy status and neuropetide Y treatment in the orange-spotted grouper Epinephelus coioides. Mol Cell Endocrinol. 2013; 376: 114–124. doi: 10.1016/j.mce.2013.06.020 PMID: 23806557

58. Pfannkuche H, Gäbel G. Glucose, epithelium, and enteric nervous system: dialogue in the dark. J Anim Physiol Anim Nutr. 2009; 93: 277–286. doi: 10.1111/j.1439-0396.2008.00847.x PMID: 19646102

59. Nakagawa Y, Nagasawa M, Yamada S, Hara A, Mogami H, Nikolakev VO, Lohse MJ, Shigemura N, Ninomiya Y, Kojima I. Sweet taste receptor expressed in pancreatic β-cells activates the calcium and cyclic AMP signaling systems and stimulates insulin secretion. PLoS ONE. 2009; 4: e5106. doi: 10.1371/journal.pone.0005106 PMID: 19352508

60. Kyriazis GA, Smith KR, Tyrberg B, Hussain T, Pratley RE. Sweet taste receptors regulate basal insulin secretion and contribute to compensatory insulin hypersecretion during the development of diabetes in male mice. Endocrinology. 2014; 155: 2112–2121. doi: 10.1210/en.2013-2015 PMID: 24712876

61. Jensen VFH, Ørgh IB, Lykkesfeldt J. Effect of insulin-induced hypoglycaemia on the central nervous system: evidence from experimental studies. J Neuroendocrinol. 2014; 26: 123–150. doi: 10.1111/jne.12133 PMID: 24428753

62. Sala-Rabanal M, Gallardo MA, Sánchez J, Pianas JM. Na-dependent D-glucose transport by intestinal brush border membrane vesicles from gilthead sea bream (Sparus aurata). J Membrane Biol. 2004; 201: 85–96.

63. Conde-Sieira M, Librán-Pérez M, López Patiño MA, Míguez JM, Soengas JL. CRF treatment induces a readjustment in glucosensing capacity in the hypothalamus and hindbrain of rainbow trout. J Exp Biol. 2011; 214: 3887–3894. doi: 10.1242/jeb.061564 PMID: 22031754