CrossMark

ORIGINAL ARTICLE

Taurine supplementation preserves hypothalamic leptin action in normal and protein-restricted mice fed on a high-fat diet

Rafael L. Camargo[1] · Thiago M. Batista[1] · Rosane A. Ribeiro[2] · Renato C. S. Branco[1] ·
Priscilla M. R. Da Silva[1] · Clarice Izumi[3] · Thiago R. Araujo[2] · Lewis J. Greene[3] ·
Antonio C. Boschero[1] · Everardo M. Carneiro[1]

Abstract Malnutrition programs the neuroendocrine axis by disruption of food-intake control, leading to obesity. Taurine (Tau) is neuroprotective and improves anorexigenic actions in the hypothalamus. We evaluated the hypothalamic gene-expression profile and food-intake control in protein-restricted mice submitted to a high-fat diet (HFD) and Tau supplementation. Mice were fed on a control (14 % protein—C) or a protein-restricted diet (6 % protein—R) for 6 weeks. Thereafter, mice received, or not, HFD for 8 weeks (CH and RH) with or without 5 % Tau supplementation (CHT and RHT). Protein restriction led to higher food intake, but calories were matched to controls. Excessive calorie intake occurred in HFD mice and this was prevented by Tau supplementation only in the CH group. Additionally, RH and CH mice developed hypothalamic leptin resistance, which was prevented by Tau. Global alterations in the expressions of genes involved in hypothalamic metabolism, cellular defense, apoptosis and endoplasmic reticulum stress pathways were induced by dietary manipulations and Tau treatment. The orexigenic peptides NPY and AgRP were increased by protein restriction and lowered by the HFD. The anorexigenic peptide Pomc was increased by HFD, and this was prevented by Tau only in CH mice. Thus, food intake was disrupted by dietary protein restriction and obesity. HFD-induced alterations were not enhanced by previous protein deficiency, but the some beneficial effects of Tau supplementation upon food intake were blunted by protein restriction. Tau effects upon feeding behavior control are complex and involve interactions with a vast gene network, preventing hypothalamic leptin resistance.

Keywords Food intake · High-fat diet · Hypothalamus · Neuropeptides · Protein restriction · Taurine supplementation

Introduction

Feeding behavior is a complex process regulated by a vast neural circuitry and associated neuropeptides, as well as by circulating nutrients, hormones and cytokines. These peripheral inputs are integrated within neuronal populations located in the arcuate nucleus (ARC) of the hypothalamus (Plum et al. 2006). The ARC contains neurons that produce the orexigenic peptides, neuropeptide Y (NPY) and agouti-related peptide (AgRP), and anorexigenic neurons that synthesize pro-opiomelanocortin (Pomc) and the cocaine- and amphetamine-regulated transcript (CART) (Munzberg and Myers 2005; Niswender et al. 2004). Signals from the ARC arrive to second-order neurons located mainly in the lateral (LH) and paraventricular (PVN) nuclei, which lead to orexigenic and anorexigenic responses, respectively, through regulation of neuropeptides and sympathetic activity (Sakurai et al. 1998; Elmquist et al. 1999; Schwartz et al. 2000; Flier 2004; Myers and Olson 2012).

✉ Rafael L. Camargo
  rlcamargo.bio@gmail.com

✉ Everardo M. Carneiro
  emc@unicamp.br

[1] Departamento de Biologia Funcional e Estrutural, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Campinas, SP CEP 13083-970, Brazil

[2] Universidade Federal do Rio de Janeiro (UFRJ), Macaé, RJ, Brazil

[3] Centro de Química de Proteínas, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (USP), Ribeirão Preto, SP, Brazil

The adipocytokine, leptin, crosses the blood–brain barrier (BBB) and acts on the hypothalamus by increasing the synthesis of anorexigenic neuropeptides and suppressing the orexigenic neuropeptides, which results in the inhibition of food intake (Jequier 2002; Dhillon and Belsham 2011). Obesity is a worldwide epidemic that contributes to the development of insulin resistance and type 2 diabetes (T2D) (Kahn et al. 2006). The disruption of the actions of the central nervous system (CNS) may contribute to obesity onset (Purkayastha and Cai 2013). In rodents submitted to high-fat diet (HFD), the hypothalamic anorexigenic effect of insulin and leptin is diminished (Münzberg et al. 2004; De Souza et al. 2005; Prada et al. 2005; Moraes et al. 2009). A HFD has shown to increase inflammatory markers in the hypothalamus that impair food intake control, contributing to adiposity (Münzberg et al. 2004; De Souza et al. 2005; Zabolotny et al. 2008).

The intrauterine period and early life are critical time-windows in which neuroendocrine axes may be programmed by environmental stressors, such as nutrient shortage (Remacle et al. 2007). As such, pups born to dams fed on a low-protein diet during gestation and lactation present long-lasting perturbations in the expression profile of hypothalamic neuropeptides and these alterations are associated with increased food intake (Orozco-Solis et al. 2011). However, whether the leptin signaling disruption that occurs in obesity (Münzberg et al. 2004) underlies the effects of protein restriction upon hypothalamic neuropeptide expression profile remains uncertain.

Taurine (Tau) is a semi-essential amino acid present in high concentrations in the brain and is responsible for the maintenance of intracellular osmotic balance (L'Amoreaux et al. 2010). Tau suppresses the activation of the endoplasmic reticulum (ER) stress response in the CNS (Pan et al. 2010; Gharibani et al. 2013). In the hypothalamus, Tau decreases NPY expression and enhances the anorexigenic effects of insulin (Solon et al. 2012). In the periphery, Tau also improves insulin secretion (Ribeiro et al. 2009; Batista et al. 2012), increases insulin receptor phosphorylation (Camargo et al. 2013), and prevents obesity (Tsuboyama-Kasaoka et al. 2006; Batista et al. 2013b). Here, using a microarray analysis, we found that several genes involved in the hypothalamic control of food intake are altered in protein-restricted mice. The association of protein restriction and HFD also changed the profile of hypothalamic mRNA expression. These alterations were associated with hypothalamic leptin resistance, which impairs food intake control in protein-restricted mice that are fed on a HFD diet. Tau supplementation prevents the disruption of leptin signaling induced by HFD and modulates the expression of several hypothalamic genes.

Materials and methods

Animals and diets

All experiments were approved by the ethics committee at UNICAMP (certificate no.: 2826-1). Weaned 30-day-old male C57Bl/6 mice were obtained from the breeding colony at UNICAMP and maintained at 21 ± 2 °C on a 12 h light–dark cycle with free access to food and water. The mice were randomly assigned to the following groups: (a) Control mice fed on a normo-protein diet (14 % protein) for 14 weeks (C group, $n = 20$); (b) protein-restricted mice fed on a diet containing 6 % protein for 14 weeks (R group, $n = 18$); (c) C mice submitted to HFD during the last 8 weeks of experimental period (CH group, $n = 20$); (d) R mice submitted to HFD during the last 8 weeks of the treatment (RH group, $n = 20$); (e) CH mice supplemented with 5 % Tau (CHT group, $n = 19$); or (f) RH-supplemented mice (RHT group, $n = 19$). Tau was administered in the drinking water from weaning until the end of the experiment. Diets were prepared according to AIN-93 guidelines (Reeves 1997), using casein as a protein source and were purchased from PRAGSOLUÇÕES Biociências© (Jaú, SP, BRA). All diet compositions have been previously described (Batista et al. 2013b).

Biometric and nutritional parameters

During the 8 weeks of HFD treatment, mice from each group were placed in metabolic cages and had their food intake and body weight (BW) measured (Batista et al. 2013b). Food intake was registered three times per week and then the mean daily food intake was determined and expressed either as g/BW/day or Kcal/day (Bachmanov et al. 2002; Bol et al. 2009; Batista et al. 2013b). At the end of the experimental period (15th week), after 12 h of fasting, the final BW and nasoanal length were measured to obtain the Lee Index [from the ratio of BW $(g)^{1/3}$/nasoanal length (cm) × 1000] (Bernardis and Patterson 1968) in all groups of mice. Subsequently, all mice were euthanized by decapitation and the retroperitoneal and perigonadal fat pads and brains were removed and weighed. Blood was collected in heparinized tubes (1:1000) and centrifuged at 2655g for 15 min, and the plasma was stored at −20 °C. Commercial kits were used according to the manufacturer's instructions for measurements of plasma triglycerides (TG), cholesterol (CHOL) (Roche/Hitachi; Indianapolis, USA), and total protein concentrations (Laborlab, Guarulhos, SP, BRA). Plasma glucose was measured using a glucose analyzer (Accu-Chek Performa, Roche Diagnostic, Switzerland), and insulin was measured by radioimmunoassay using human insulin radiolabelled with ^{125}I as tracer, rat insulin as standard (Crystal Chem. Inc., Downers Grove, IL, USA), and rat insulin

antibody (donated by Dr Leclerq-Meyer, Free University of Brussels, Brussels, Belgium). The charcoal–dextran method was used to separate free insulin from antibody bound to ^{125}I-insulin (Ribeiro et al. 2010). The limits for detection ranged from 0.02 to 5 ng/mL and the coefficient of variation was lower than 10 %.

TAU content in the hypothalamus

The analysis of TAU content in the hypothalamus was performed with reverse-phase HPLC method. The hypothalamus was disrupted by sonication in 150–200 μL of a solution containing 0.2 M perchloric acid, 0.01 M EDTA, and 100 mM alfa-aminobutyric acid as an internal standard. The homogenates were centrifuged at 20,000g at 4 °C for 15 min. An aliquot of the supernatant (10 μL) was dried in SpeedVac, and derivatized with phenylisothiocyanate (Bidlingmeyer et al. 1987). The phenylthiocarbamoyl–amino acid derivatives in each sample were resuspended in 0.14 M sodium acetate buffer containing 0.05 % triethylamine, pH 7.5, and then separated using a LDC MP3000 HPLC binary system (Milton Roy Co., Riviera Beach, FL, USA) and Picotag C_{18} 5 μm column, 3.9 × 150 mm (Waters Corporation, Milford, MA, USA) and detected by UV (254 nm). Elution was performed at 1 ml/min at 38 °C using a gradient of 0.14 M sodium acetate, 0.05 % triethylamine and pH 5.7 as solution A, and acetonitrile/H_2O (60:40, v/v) as solution B. Amino acid standards (Standard H from Pierce Protein Reagent Products, Pittsburgh, PA, USA) were derivatized and analyzed together with the samples. The amino acid peak identification was carried out by the amino acid retention time, and area integration by LCTalk software (Thermo Separation Products). Amino acid content for hypothalamus was expressed as nmol/mg hypothalamus weight.

Microarray analysis

Global hypothalamic gene expression was measured using the microarray, GeneChip® Mouse Genome 430 2.0 Array, from Affymetrix® Company (Santa Clara, CA, USA). The array contains 45,000 probe sets that analyze the expression level of over 39,000 transcripts and variants from over 34,000 well-characterized mouse genes. After decapitation, mice brains were removed from the skull and placed in a ventral position. The entire hypothalamus was dissected away from the optic chiasm (anterior) and mammillary bodies (posterior) and total RNA was extracted using Trizol® (Invitrogen, São Paulo, SP, BRA). A total of 200 ng RNA was used for cDNA synthesis, which was processed with the 3′ IVT Express Kit Assay. The hybridization, washing, and revelation steps with streptavidin-PE were performed using the Hybridization, Wash, and Stain kit,

according to the manufacturer's instructions. The arrays were analyzed using an Affymetrix® GeneChip® Scanner 3000 (Affymetrix, Santa Clara, CA, USA) at the DNA microarray facility (LMA), Brazilian Synchrotron Light Laboratory (Campinas, SP, BRA). Scanned images were analyzed in the R environment using the Affy and Rank-Prod packages from Bioconductor using the algorithm MAS 5. We normalized the arrays and then calculated the signal intensity and the detection (the presence or absence of expression). For statistical analysis, we used the Rank Product test, which is biologically motivated and designed to test and detect differentially expressed genes in replicated microarray experiments. This is a simple non-parametric statistical method based on ranks of fold changes. We used a P value <0.04 and a percentage of false positives (pfp) < 0.1, in order to consider a given transcript as differentially expressed. The number of probe sets in each comparison is shown in Table 3. Pathway enrichment analyses were conducted using the MetaCoreTM platform (http://www.genego.com/metacore.php, GeneGo, St. Joseph, MI, USA) (Gentleman et al. 2004; Gautier et al. 2004; Team 2011; Hong et al. 2013).

Real time RT-PCR

The microarray analysis of the hypothalamic neuropeptides was confirmed by real time quantitative RT-PCR. The hypothalamic mRNA extraction was performed using Trizol®, according to the manufacturer's instructions (Invitrogen, São Paulo, SP, BRA). A sample of 2 μg of each RNA was used for each reverse transcription reaction (RT) with random primers using DTT (100 mM), a dNTP mix (10 mM), and the enzyme SuperScript II (200U; Invitrogen, São Paulo, SP, BRA). The samples were then incubated for 50 min at 42 °C to obtain the cDNAs. The PCR reactions were completed with a final volume of 20 μL and contained 4 μL cDNA (0.1 μg/μL), 10 pM of NPY (Forward 5′TACTCCGCTCTGCGACACTA3′, Reverse 5′TCTTCAAGCCTTGTTCTGGG3′), or AgRP (Forward 5′GAGTTCCCAGGTCTAAGTCTGAATG3′, Reverse 5′ATCTAGCACCTCCGCCAAAG3′), or Pomc (Forward 5′GGCTTGCAAACTCGACCTC3′, Reverse 5′TGACCCATGACGTACTTCCG3′) or CART (Forward 5′ACCTTTGCTGGGTGCCCGTG3′, Reverse 5′TGCAACGCTTCGATCAGCTCC3′) primers and SYBR Green PCR Master Mix (Applied Biosystems, São Paulo, SP, BRA). The transcripts were detected using the 7000 real time PCR system (Applied Biosystems, São Paulo, SP, BRA), and the threshold cycle values were used to calculate the number of cycles required to first detect a statistically significant increase in fluorescence intensity due to the binding of SYBR Green to a molecule of double-stranded DNA. The values were normalized by the values

of control gene, rlpl0 (ribosomal protein large P0, or 36B4) (Forward 5′GAGGAATCAGATGAGGGATATGGGA3′, Reverse 5′AAGCAGGCTGACTTGGTTGC3′) and were shown as relative expression over the control group level (2-ΔΔCT).

Evaluation of leptin action on food intake

During the last week of treatment (14th week), mice were acclimatized in metabolic cages for 24 h and fasted for 6 h, from 1:00 to 7:00 p.m., when feeding activity is low (Orozco-Solis et al. 2011). At the end of this period, an intraperitoneal (i.p.) injection of saline (0.9 % NaCl) was administered and food was made available. The same protocol was repeated for two consecutive days. Food intake was measured 12 h after saline administration on each day and the average of two consecutive days was determined. On the fourth and fifth days, the same mice were fasted for 6 h (1:00 to 7 p.m.) before receiving a single i.p. injection of leptin (500 ng/g BW; recombinant mouse leptin from ©Merck—Calbiochem cat. #429705, Darmstadt, Germany) according to (Vieira et al. 2012) with minor modifications. Food intake was measured 12 h after leptin administration and the average of two consecutive days was determined.

Western blot

The hypothalamic leptin action was evaluated in mice from all groups. After evaluation of food intake on the second day of leptin administration, mice were fasted for 1 h before another leptin load for hypothalamic leptin signaling assessment. Conscious mice received an i.p. injection of leptin (500 ng/g BW) or saline (0.9 % NaCl). After 45 min (without food access), the mice were euthanized by decapitation, and the hypothalamus was removed and immediately homogenized in antiprotease buffer containing 100 mM Tris pH 7.5, 10 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium vanadate, 2 mM PMSF, and 1 % Triton X-100. The extracts were then centrifuged at 15,294g at 4 °C for 40 min to remove insoluble material. The protein concentration was determined by the Bradford dye method (Bradford 1976), using bovine serum albumin as a standard curve. For PAGE and Western blot analysis, the samples were treated with a Laemmli sample buffer containing dithiothreitol. After heating to 95 °C for 5 min, the proteins were separated by electrophoresis (70 µg protein/lane, 10 % gels). Following electrophoresis, the proteins were transferred to nitrocellulose membranes. The membranes were treated with a blocking buffer (5 % non-fat dried milk, 10 mM Tris, 150 mM NaCl, and 0.02 % Tween 20) and were subsequently incubated overnight with a polyclonal antibody against the phospho-signal transducer and activator of transcription 3 (pSTAT3;

1:1000, cat. #9131S) or total STAT3 (1:1000, cat. #4904S). Both primary antibodies were from Cell Signaling Technology® (Danvers, MA, USA). Detection was performed after a 2 h incubation period using a horseradish peroxidase-conjugated secondary antibody (1:10,000, Invitrogen, São Paulo, SP, BRA). The band intensities were quantified by optical densitometry using the free software Image J Tool (http://ddsdx.uthscsa.edu/dig/itdesc.html). The densitometry values obtained from pSTAT3 were normalized by STAT3 protein expression. After assaying the target proteins, Western blotting was repeated using a rabbit polyclonal antibody to the glyceraldehyde 3-phosphate dehydrogenase protein (GAPDH; 1:1000; cat. no sc-25778, Santa Cruz Biotechnologies, CA, USA) as an internal control.

Statistical analysis

The results are presented as mean ± SEM for the number of samples (n) indicated. Statistical analyses were carried out using a Student's t test (to compare results from saline and leptin-stimulated conditions, Fig. 3) or one-way analysis of variance (ANOVA) followed by Duncan's post-test with the Statistica 5.0 software (Stantsoft, Tulsa, OK, USA), and the level of significance was set at $P < 0.05$. Prior to ANOVA analysis, a Gaussian distribution of the samples was assumed based on the Kolmogorov–Smirnov normality test using Graph-Pad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Metabolic characterization

At the end of the experimental period, R mice showed a lower body weight and nasoanal length (BW) compared with C mice ($P < 0.05$; Table 1). The HFD treatment increased BW and body length in the CH and RH groups ($P < 0.05$). Tau supplementation reduced BW in the CHT group, when compared with the CH mice ($P < 0.05$). HFD efficiently induced obesity, as the CH and RH groups showed a higher Lee index and retroperitoneal and perigonadal fat pads in comparison with the C and R groups, respectively ($P < 0.05$; Table 1). Tau supplementation decreased fat deposition only in the CHT mice ($P < 0.05$), but the treatment efficiently normalized the Lee index in both the RHT and CHT groups (Table 1). The R mice exhibited an enhanced ratio between brain weight and BW, when compared with the C mice ($P < 0.05$). All HFD-fed groups, regardless of Tau supplementation, showed a decrease in the brain/BW ratio in comparison with their respective controls ($P < 0.05$;

Table 1 Final body weight (BW), nasoanal length, Lee index, retroperitoneal (RP), and perigonadal (PG) fat pads, brain weight, and hypothalamic Tau concentration in C, CH, CHT, R, RH, RHT mice

	C	CH	CHT	R	RH	RHT
BW (g)	26.71 ± 0.53	38.00 ± 1.22*	33.00 ± 1.53*,&	20.76 ± 1.33*	29.84 ± 0.92#	29.96 ± 1.14#
Nasoanal lenght (cm)	9.08 ± 0.13	9.67 ± 0.12*	9.95 ± 0.12*	8.69 ± 0.10*	9.31 ± 0.11#	9.78 ± 0.11*,#,$
Lee index	315 ± 3	334 ± 4*	320.70 ± 1.53&	313.4 ± 1.22	326.20 ± 3.30*,#	$316.10 \pm 2.33$$
RP fat pad (% BW)	0.52 ± 0.03	1.41 ± 0.04*	1.11 ± 0.03*,&	0.63 ± 0.04	1.13 ± 0.06*,#	1.03 ± 0.06*,#
PG fat pad (% BW)	1.43 ± 0.06	4.12 ± 0.18*	3.14 ± 0.20*,&	1.79 ± 0.09	2.50 ± 0.16*,#	2.49 ± 0.22*,#
Brain (% BW)	1.56 ± 0.03	1.13 ± 0.04*	1.26 ± 0.04*	2.02 ± 0.11*	1.42 ± 0.04#	1.41 ± 0.05#
Tau (nmol/mg hypothalamus)	19.75 ± 3.41	21.85 ± 3.86	37.22 ± 2.52*,&	32.81 ± 1.67*	36.27 ± 2.42*	55.40 ± 1.51*,#,$

Data are mean $\pm$ SEM ($n = 4$–15)

One-way ANOVA followed by Duncan's post-test ($P < 0.05$)

* Different from C; & different from CH; # different from R; $ different from RH

Table 2 Biochemical nutritional parameters analyzed in fasted and fed C, CH, CHT, R, RH, and RHT mice

	C	CH	CHT	R	RH	RHT
Glucose (mg/dL)						
Fasted	75 ± 3	95 ± 6*	80 ± 4&	74 ± 5	89 ± 5#	89 ± 4#
Fed	139 ± 10	157 ± 12	151 ± 9	135 ± 6	144 ± 4	144 ± 4
Insulin (ng/mL)						
Fasted	0.44 ± 0.09	1.30 ± 0.20*	0.61 ± 0.13&	0.31 ± 0.08	0.39 ± 0.09	0.40 ± 0.15
Fed	1.31 ± 0.36	2.87 ± 0.49*	1.87 ± 0.28&	0.97 ± 0.22	1.51 ± 0.21	1.70 ± 0.30
TG (mg/dL)						
Fasted	92 ± 6	105 ± 4	109 ± 5	96 ± 7	102 ± 5	109 ± 5
Fed	113 ± 12	154 ± 26	183 ± 27	86 ± 19	171 ± 14#	233 ± 34*,#
CHOL (mg/dL)						
Fasted	93 ± 10	158 ± 14*	125 ± 10*,&	91 ± 6	142 ± 8*,#	153 ± 7*,#
Fed	113 ± 9	198 ± 18*	236 ± 12*	113 ± 12	206 ± 11*,#	235 ± 11*,#
Total proteins (g/dL)						
Fasted	5.1 ± 0.1	5.4 ± 0.2	5.2 ± 0.2	4.5 ± 0.1*	5.1 ± 0.2#	5.1 ± 0.2#

Data are mean $\pm$ SEM ($n = 4$–10)

One-way ANOVA followed by Duncan's post-test ($P < 0.05$)

* Different from C; & different from CH; # different from R; $ different from RH

Table 1). Hypothalamus weight was not modified either by malnutrition (R 7.1 ± 0.5 and C 7.1 ± 0.6 mg), HFD (CH 8.9 ± 0.2 and RH 6.6 ± 0.9 mg) or Tau supplementation (CHT 6.3 ± 0.5 and RHT 5.9 ± 0.4 mg). Additionally, hypothalamic Tau concentration was elevated by nearly 1.8-fold in RHT and CHT mice compared with their respective controls ($P < 0.0001$; Table 1). Of note, Tau content in the hypothalamus of R mice was also significantly higher than of that observed in C hypothalamus ($P < 0.005$; Table 1).

All HFD mice showed higher fasting glycaemia, when compared with their respective controls ($P < 0.05$; Table 2). Tau treatment normalized glycaemia only in the CHT group (Table 2), whereas, under fed conditions no alterations in plasma glucose levels were observed between the groups (Table 2). In addition, only the CH mice developed hyperinsulinemia, in both the fasted and fed states, when compared with the C mice ($P < 0.05$; Table 2). Tau supplementation normalized plasma insulin in the CHT mice (Table 2). The plasma lipid profile was also modulated by the nutritional interventions. The TG levels were unaltered during fasting, but were higher in the RH and RHT fed mice when compared with R ($P < 0.05$; Table 2). The HFD treatment increased plasma CHOL in the normal and protein-restricted mice in both nutritional states ($P < 0.05$; Table 2). Tau supplementation resulted in a reduction in CHOL only in the fasted CHT mice, when compared with the CH mice ($P < 0.05$; Table 2). In addition, the low-protein diet treatment lowered total plasma proteins, in comparison with the C group ($P < 0.05$; Table 2). The HFD treatment recovered plasma proteins in all groups (Table 2).
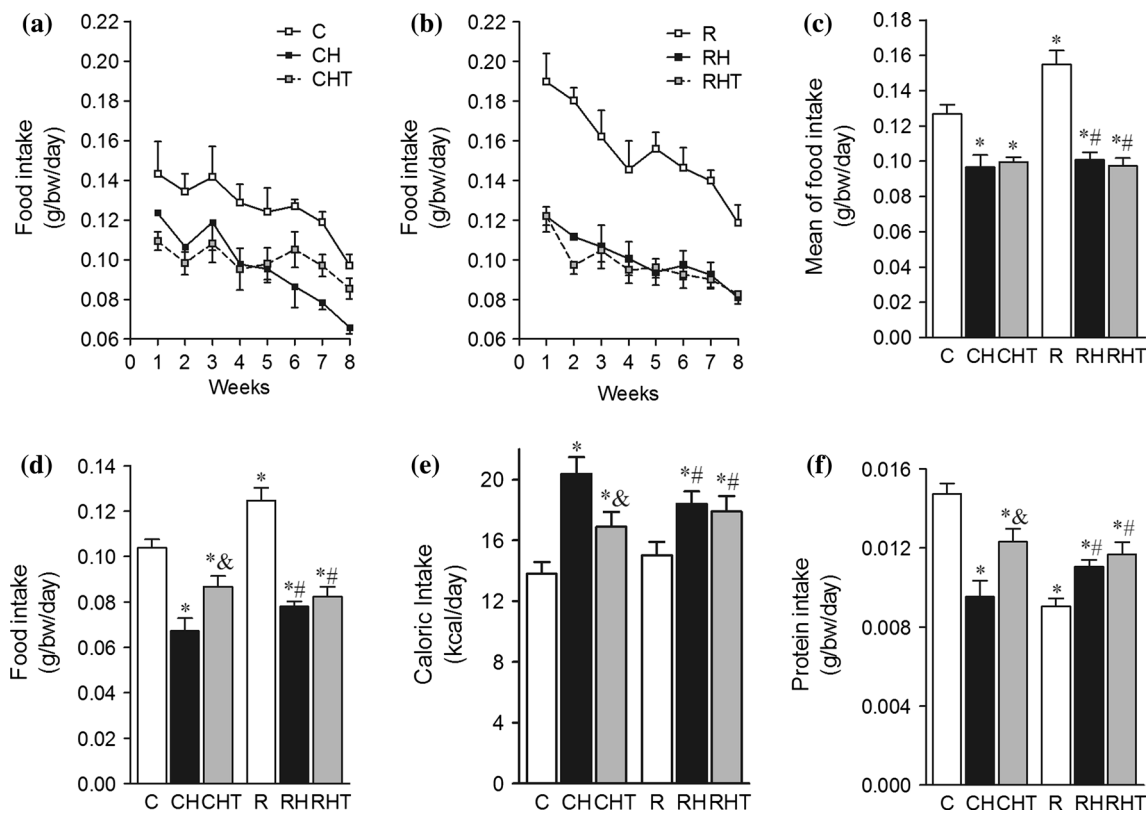
Fig. 1 Food consumption in mice undergoing protein-restriction with or without HFD and Tau supplementation. The food intake of (**a**) the C, CH, CHT and (**b**) the R, RH, RHT mice was recorded for eight weeks. Mean food intake during the HFD treatment (**c**). Food (**d**), calorie (**e**) and protein intake (**f**) recorded during the last week of the experimental period. Data are mean ± SEM ($n = 4$–8). *Asterisk* different from *C*; *ampersand* different from *CH*; *hash* different from *R*; *dollar* different from *RH*. One-way ANOVA followed by Duncan's post-test ($P < 0.05$)

Food intake

Figure 1 shows the weekly ratio between food consumption and BW. Protein-restricted mice showed enhanced food intake, as judged by mean food intake during the 8 weeks of HFD treatment, when compared with the C group ($P < 0.05$; Fig. 1c). All the HFD mice had a lower food intake than the C mice ($P < 0.05$; Fig. 1c).

During the last week of treatment, the R mice were persistently hyperphagic ($P < 0.05$; Fig. 1d), but their calorie intake was matched to that of the C mice (Fig. 1e). In addition, although the RH and CH groups showed a lower amount of food intake, when compared with their respective controls ($P < 0.05$; Fig. 1d), the calorie intake was higher due to the hypercaloric nature of the diet ($P < 0.05$; Fig. 1e). Tau supplementation modified the total food intake only in the CHT mice ($P < 0.05$; Fig. 1d) and decreased the total calorie intake, when compared with the CH mice (Fig. 1e). However, the RHT mice persistently showed a hypercaloric intake at the end of the experiment (Fig. 1e). Protein intake in the R mice remained low despite an increase in food intake (Fig. 1d, f). The low food intake

in the HFD mice also favored a lower protein intake; however, the CHT mice presented an increase in protein intake, when compared with the CH mice (Fig. 1f).

Hypothalamic gene expression profile

The altered food consumption observed in the protein-restricted mice and the hypercaloric intake observed in the RH and RHT groups suggest a deleterious action of the low-protein diet upon the hypothalamic control of food intake. Hence, we performed a microarray analysis of the hypothalamus in order to gain insight into the possible mechanisms responsible for these alterations.

The groups of mice exhibited changes in global hypothalamic gene expression. Hypothalamic genes were grouped into (1) control of food intake (Table 4); (2) tricarboxylic acid cycle (TCA), oxidative phosphorylation and glycolysis/gluconeogenesis (Table 5); (3) unfolded protein response, apoptosis, and endoplasmic reticulum (ER) stress (Table 6); and (4) signal transduction leptin/insulin signaling (Table 7).

The low-protein diet significantly changed the expression of 787 probe sets, 54 % of which were up-regulated

Table 3 General data from the Affymetrix microarray analyses performed using the GeneChip® Mouse Genome 430 2.0

Comparison between groups	Probe sets analyzed	Probe sets with a pfp < 0.1	N probe sets up-regulated	N probe sets down-regulated
R × C	2474	787	425	362
CH × C	2362	769	427	342
CHT × CH	2137	429	139	290
RH × R	2411	739	334	405
RHT × RH	2302	540	140	400

$n = 5$ chips per group. pfp < 0.1

Table 4 Genes involved in the control of food intake expressed by fold change between two groups

ID and gene name	Gene symbol	Fold change		
		Protein-restriction R × C	High-fat diet CH × C/RH × R	Taurine CHT × CH/RHT × RH
Orexigenic neuropeptides				
1419127_at neuropeptide Y	NPY	1.17	−1.22/−1.41	nsd/−1.42
1421690_s_at agouti-related protein	AgRP	1.41	−1.65/−1.89	nsd/nsd
Anorexigenic neuropeptides				
1455858_x_at pro-opiomelanocortin-alpha	Pomc	nsd	nsd/2.30	nsd/1.29
1422825_at CART prepropetide	Cartpt	−1.16	1.18/1.30	nsd/1.10

Fold change above zero represent gene up-regulation, and the fold change below zero represent gene down-regulation between two groups

$n = 5$ chips per group. pfp < 0.1

nsd not significantly different

and 46 % were down-regulated in the R mice. Furthermore, in the CH hypothalamus, the expression of 769 probe sets was modified, 56 % of which were up-regulated and 44 % down-regulated in comparison with the C group. Protein-restricted mice fed on a HFD modified the expression of 739 probe sets, with an up-regulation of 55 % and a down-regulation of 45 %, when compared with the R group. Finally, Tau supplementation altered the expression of 429 probe sets, with an up-regulation of 32 % and down-regulation of 68 % in the CHT mice, when compared with the CH group. Moreover, in the RHT mice, there were alterations in 540 probe sets, where 26 % were up-regulated and 74 % down-regulated, when compared with the RH group (Table 3).

Microarray analysis showed that hypothalamic neuropeptides involved in the control of food intake are regulated by diet and/or Tau treatment (Table 4). In the R group, NPY and AgRP gene expressions were higher, when compared with the C group ($P < 0.0004$ and $P < 0.04$; Table 4). Both the CH and RH mice showed a significant decrease in these orexigenic neuropeptides, when compared with the C mice ($P < 0.0002$ and $P < 0.0001$) and R mice ($P < 0.0001$), respectively (Table 4). Tau supplementation decreased the NPY mRNA content in the RHT group, when compared with the RH mice ($P < 0.0001$; Table 4), whereas it did not modify AgRP mRNA expression between the other groups.

In addition, in the R mice, there was a decrease in CART prepropeptide (CARTpt) expression, when compared with the C mice ($P < 0.0001$), without any alteration in Pomc mRNA. Protein-restricted mice fed on a HFD diet showed a higher expression of Pomc and CARTpt genes, in comparison with the R group ($P < 0.0001$; Table 4). In the CH mice, only the expression of CARTpt was increased ($P < 0.0007$). Tau supplementation increased the gene expression of these anorexigenic neuropeptides only in the RHT group (Table 4).

In addition to genes related to food intake, we also found that protein restriction, HFD and/or Tau treatment modified the expression of several genes involved in different metabolic pathways in the hypothalamus (Tables 5, 6, 7).

The genes involved in the tricarboxylic acid cycle (TCA), glycolysis/gluconeogenesis, and oxidative phosphorylation are shown in Table 5. Microarray analysis showed that 6 genes that play a role in glycolysis/gluconeogenesis were down-regulated in the R group, when compared with the C group (Table 5). In the hypothalamus from CH mice, 4 genes were up-regulated and 4 down-regulated. In the RH group, the mRNA content for 2 genes was up-regulated and for 4 genes was reduced (Table 5). Tau supplementation decreased Aldoc, but enhanced Mdh1, Aldoa, and Tpi1 in CHT, when compared with CH (Table 5). For oxidative phosphorylation, 15 genes were down-regulated

Table 5 Hypothalamic genes involved in tricarboxylic acid cycle, glycolysis/gluconeogenesis, and oxidative phosphorylation expressed by fold change between two groups

ID and gene name	Gene symbol	Fold change		
		Protein-restriction R×C	High-fat diet CH × C/RH × R	Taurine CHT × CH/RHT × RH
Tricarboxylic acid cycle				
1433984_a_at malate dehydrogenase 2. NAD (mitochondrial)	Mdh2	−1.07	−1.04/1.02	nsd/nsd
1432016_a_at isocitrate dehydrogenase 3 (NAD+) alpha	Idh3a	nsd	1.11/nsd	nsd/1.09
1450667_a_at citrate synthase	Cs	nsd	nsd/nsd	1.06/nsd
Glycolysis and gluconeogenesis				
1451461_a_at aldolase C. fructose-bisphosphate	Aldoc	−1.02	1.00/−1.00	−1.06/−1.03
1433984_a_at malate dehydrogenase 2. NAD (mitochondrial)	Mdh2	−1.07	−1.04/1.02	nsd/nsd
1434799_x_at aldolase A. fructose-bisphosphate	Aldoa	−1.05	nsd/nsd	1.06/nsd
1454925_x_at malate dehydrogenase 1. NAD (soluble)	Mdh1	−1.03	1.00/−1.06	1.04/1.08
1434814_x_at glucose phosphate isomerase 1	Gpi1	−1.06	−1.05/nsd	nsd/nsd
1426554_a_at phosphoglycerate mutase 1	Pgam1	1.08	nsd/−1.07	nsd/nsd
1435659_a_at triosephosphate isomerase 1	Tpi1	nsd	−1.08/nsd	1.08/nsd
1416183_a_at lactate dehydrogenase B	Ldhb	nsd	1.04/−1.06	nsd/1.07
Oxidative phosphorylation				
1426088_at predicted gene 4076	Gm4076	−1.10	1.08/1.23	−1.11/−1.14
1430713_s_at NADH dehydrogenase (ubiquinone) 1 alpha subcomplex. 13	Ndufa13	−1.14	nsd/1.07	nsd/nsd
1416970_a_at cytochrome c oxidase. subunit VIIa 2	Cox7a2	−1.07	−1.04/1.02	1.04/1.02
1433513_x_at NADH dehydrogenase (ubiquinone) 1 alpha subcomplex. 12	Ndufa12	−1.06	nsd/nsd	nsd/nsd
1456580_s_at ATP synthase. H+ transporting. mitochondrial F1 complex. delta subunit	Atp5d	−1.11	nsd/nsd	nsd/1.05
1456588_x_at cytochrome c oxidase. subunit Vb	Cox5b	−1.07	nsd/−1.02	nsd/102
1423907_a_at NADH dehydrogenase (ubiquinone) Fe-S protein 8	Ndufs8	−1.13	1.07/1.16	−1.08/−1.13
1423111_at ATP synthase. H + transporting. mitochondrial F1 complex. alpha subunit 1	Atp5a1	1.08	1.09/−1.06	nsd/nsd
1428464_at NADH dehydrogenase (ubiquinone) 1 alpha subcomplex. 3	Ndufa3	−1.05	−1.02/nsd	1.06/1.05
1416829_at ATP synthase. H+ transporting mitochondrial F1 complex. beta subunit	Atp5b	1.05	nsd/−1.03	1.06/nsd
1434491_a_at cytochrome c oxidase. subunit Vic	Cox6c	−1.09	−1.10/−1.06	nsd/nsd
1422484_at cytochrome c. somatic	Cycs	−1.09	nsd/1.07	nsd/nsd
1415933_a_at cytochrome c oxidase. subunit Va	Cox5a	−1.03	−1.05/−1.03	1.04/nsd
1422525_at ATP synthase. H+ transporting. mitochondrial F1F0 complex. subunit e	Atp5 k	−1.05	nsd/1.03	nsd/nsd
1429708_at NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 11	Ndufa11	−1.10	−1.17/nsd	nsd/1.12
1452133_at ubiquinol-cytochrome c reductase hinge protein	Uqcrh	1.03	1.07/1.07	−1.00/nsd
1423676_at ATP synthase. H + transporting. mitochondrial F0 complex. subunit d	Atp5 h	−1.02	1.01/1.05	1.05/1.01
1438159_x_at NADH dehydrogenase (ubiquinone) flavoprotein 2	Ndufv2	1.08	1.12/nsd	nsd/nsd
1424085_at NADH dehydrogenase (ubiquinone) 1 alpha subcomplex. 4	Ndufa4	nsd	1.07/1.03	−1.00/nsd

Table 5 continued

ID and gene name	Gene symbol	Fold change		
		Protein-restriction R×C	High-fat diet CH × C/RH × R	Taurine CHT × CH/RHT × RH
1448934_at NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 10	Ndufa10	nsd	1.08/nsd	nsd/nsd
1428075_at predicted pseudogene 3244///NADH dehydrogenase (ubiquinone) 1 beta subcomplex 4	Gm3244///Ndufb4	nsd	−1.04/−1.06	nsd/nsd
1424628_a_at NADH dehydrogenase (ubiquinone) flavoprotein 3	Ndufv3	nsd	1.06/1.07	nsd/nsd
1450968_at ubiquinol-cytochrome c reductase. Rieske iron-sulfur polypeptide 1	Uqcrfs1	nsd	1.11/nsd	nsd/nsd
1448284_a_at NADH dehydrogenase (ubiquinone) 1. subcomplex unknown. 1	Ndufc1	nsd	1.05/nsd	−1.02/nsd
1428631_a_at ubiquinol cytochrome c reductase core protein 2	Uqcrc2	nsd	1.06/nsd	nsd/nsd
1448198_a_at NADH dehydrogenase (ubiquinone) 1 beta subcomplex 8	Ndufb8	nsd	−1.04/−1.05	nsd/nsd
1452184_at NADH dehydrogenase (ubiquinone) 1 beta subcomplex. 9	Ndufb9	−1.05	−1.08/−1.05	1.06/1.05

Fold change above zero represents gene upregulation, and fold change below zero represents gene down-regulation between the two compared groups

$n = 5$ chips per group. pfp < 0.1

nsd not significantly different

by protein restriction (Table 5). The HFD treatment reduced the expressions of 8 genes and increased the expressions of 12 mRNAs in the hypothalamus of CH mice. In RH, 8 genes were down-regulated and 10 genes were up-regulated (Table 5). Tau prevented the HFD-induced alterations in genes involved in oxidative phosphorylation, as 9 genes showed an inverted pattern of expression, compared with the CH group (Table 5). Supplementation in the RHT group only reversed the expression of 6 genes (Table 5).

Genes involved in the unfolded protein response, apoptosis, and ER stress pathway were also modified between the groups and are shown in Table 6. Among the 23 genes involved in these processes that were modified by diet and/or Tau treatment, in the hypothalamus of R mice, 10 were down-regulated and 7 were up-regulated, when compared with the C mice (Table 6). Twelve genes were up-regulated and a decrease in 1 gene occurred in CH mice. In RH mice, 6 genes were up-regulated and 4 genes were reduced (Table 6). Tau treatment reversed the HFD-induced gene expression alteration in 7 mRNAs in the CH and 6 mRNAs in the RH mice that are involved in unfolded protein response, apoptosis, and ER stress pathway (Table 6).

The leptin signaling via PI3 K-dependent pathway and inflammation JAK-STAT pathway were altered in hypothalamus by diet and/or Tau supplementation and are shown in Table 7. In the R group, 15 genes involved in these pathways were up-regulated and 4 reduced in comparison with the C group (Table 7). HFD consumption lowered the content of 10 and 13 hypothalamic genes in the CH and in the RH group, respectively (Table 7). Tau supplementation reversed the expressions of 6 genes in the CHT mice and 2 genes in RHT mice (Table 7).

Gene expression of neuropeptides involved in feeding behavior

Figure 2 shows the gene expressions of the orexigenic and anorexigenic neuropeptides that were detected at significant levels in microarray analyses and were modified by diet and/or Tau supplementation. The hypothalamus from the R mice presented increases of 37 and 60 % in NPY and AgRP gene expressions, respectively, when compared with the C group ($P < 0.05$; Fig. 2a, b. Conversely, in the CH mice, reductions in NPY and AgRP of 46 and 42 %, respectively, were observed compared with the C mice ($P < 0.05$; Fig. 2a, b). In the RH group, there was a 31 % decrease in the mRNA content of NPY and AgRP compared with the R mice ($P < 0.05$; Fig. 2a, b). Tau did not alter the HFD-induced alterations in the expression of orexigenic neuropeptides (Fig. 2a, b).

The mRNA content of Pomc and CART did not differ between the R and C mice (Fig. 2c, d). HFD treatment increased the expressions of Pomc and CART in the CH mice ($P < 0.05$; Fig. 2c, d). In the RH mice, no modifications in anorexigenic neuropeptides were observed in the hypothalamus. Tau supplementation did not modify these

Table 6 Hypothalamic genes involved in unfolded protein response, apoptosis, and ER stress pathway expressed by fold change between two groups

ID and gene name	Gene symbol	Fold change		
		Protein-restriction R × C	High-fat diet CH × C/RH × R	Taurine CHT × CH/RHT × RH
Protein folding response to unfolded protein/apoptosis ER stress pathway/protein folding ER cytoplasm				
1425993_a_at heat shock 105 kDa/110 kDa protein 1	Hsph1	−1.22	1.09/nsd	1.16/nsd
1420622_a_at heat shock protein 8	Hspa8	1.14	1.09/−1.05	−1.04/1.00
1416064_a_at heat shock protein 5	Hspa5	−1.06	1.10/nsd	−1.15/1.03
1416365_at heat shock protein 90 alpha (cytosolic). class B member 1	Hsp90ab1	1.03	1.13/1.10	−1.35/−1.18
1438040_a_at heat shock protein 90. beta (Grp94). member 1	Hsp90b1	1.16	1.23/nsd	−1.35/−1.19
1416755_at DnaJ (Hsp40) homolog. subfamily B. member 1	Dnajb1	−1.27	nsd/nsd	nsd/nsd
1452318_a_at heat shock protein 1B	Hspa1b	−2.46	1.96/nsd	nsd/−2.84
1437497_a_at heat shock protein 90. alpha (cytosolic). class A member 1	Hsp90aa1	−1.02	1.09/1.14	−1.25/−1.21
1427442_a_at amyloid beta (A4) precursor protein	App	−1.00	1.07/1.07	−1.02/−1.08
1456170_x_at Calreticulin	Calr	−1.10	nsd/nsd	nsd/nsd
1437223_s_at X-box binding protein 1	Xbp1	Nsd	1.10/1.06	−1.09/nsd
1416288_at DnaJ (Hsp40) homolog. subfamily A. member 1	Dnaja1	Nsd	1.11/nsd	nsd/nsd
1454664_a_at eukaryotic translation initiation factor 5	Eif5	1.18	nsd/1.08	nsd/−1.26
1426205_at similar to protein phosphatase 1/// protein phosphatase 1. catalytic subunit. beta isoform	LOC100044953///Ppp1cb	1.11	nsd/−1.07	nsd/−1.03
1437195_x_at mitogen-activated protein kinase 10	Mapk10	−1.07	nsd/nsd	nsd/nsd
1422484_at cytochrome c. omatic	Cycs	−1.09	nsd/1.07	nsd/nsd
1426401_at protein phosphatase 3. catalytic subunit. alpha isoform	Ppp3ca	1.06	1.06/nsd	nsd/nsd
1415859_at eukaryotic translation initiation factor 3. subunit C	Eif3c	1.26	nsd/nsd	−1.33/−1.30
1452662_a_at eukaryotic translation initiation factor 2. subunit 1 alpha	Eif2s1	−1.20	nsd/nsd	nsd/nsd
1423798_a_at eukaryotic translation initiation factor 1	Eif1	Nsd	−1.05/−1.15	nsd/1.05
1450149_a_at protein phosphatase 1. catalytic subunit. gamma isoform	Ppp1cc	Nsd	nsd/nsd	−1.05/1.05
1420088_at nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor. Alpha	Nfkbia	Nsd	nsd/nsd	nsd/−1.16
1416858_a_at FK506 binding protein 3	Fkbp3	nsd	nsd/−1.05	nsd/nsd

Fold change above zero represents gene up-regulation, and fold change below zero represents gene down-regulation between the two compared groups

$n = 5$ chips per group. pfp < 0.1

nsd not significantly different

parameters in the CHT group (Fig. 2c, d); however, Pomc and CART mRNA were significantly increased in the RHT group ($P < 0.05$; Fig. 2c, d). It is important to highlight that the control gene rlpl0 was not altered by either dietary or Tau interventions.

Leptin-induced anorexigenic response and hypothalamic signaling

Finally, the hypothalamic control of food consumption was evaluated using leptin in all groups (Fig. 3). Leptin

Table 7 Hypothalamic genes involved in leptin and insulin signaling, and the leptin signaling via PI3 K-dependent pathway/inflammation JAK-STAT pathway, expressed as fold change difference between two groups

ID and gene name	Gene symbol	Fold change		
		Protein-restriction R × C	High-fat diet CH × C/RH × R	Taurine CHT × CH/RHT × RH
Signal transduction leptin/insulin signaling				
1443798_at phosphatidylinositol 3-kinase catalytic delta polypeptide	Pik3 cd	1.00	−1.16/−1.06	−1.00/−1.03
1420611_at protein kinase. cAMP dependent. catalytic. Beta	Prkacb	1.08	1.07/nsd	−1.15/−1.10
1452032_at protein kinase. cAMP dependent regulatory. type I. alpha	Prkar1a	1.07	1.04/nsd	nsd/nsd
1422484_at cytochrome c. somatic	Cycs	−1.09	nsd/1.07	nsd/nsd
1447720_x_at protein kinase. cAMP dependent. catalytic. Alpha	Prkaca	1.03	nsd/nsd	nsd/nsd
1437689_x_at clusterin///similar to clusterin	Clu///LOC100046120	−1.03	−1.05/−1.04	nsd/nsd
1434325_x_at protein kinase. cAMP dependent regulatory. type I beta	Prkar1b	−1.03	−1.02/nsd	nsd/nsd
1435638_at glycogen synthase kinase 3 alpha	Gsk3a	nsd	−1.27/nsd	nsd/nsd
1417065_at early growth response 1	Egr1	nsd	−1.18/−1.18	nsd/−1.29
1437001_at glycogen synthase kinase 3 beta	Gsk3b	nsd	nsd/nsd	−1.29/−1.22
1455858_x_at pro-opiomelanocortin-alpha	Pomc	nsd	nsd/2.30	nsd/1.30
1423100_at FBJ osteosarcoma oncogene	Fos	nsd	nsd/−1.47	nsd/nsd
1421867_at nuclear receptor subfamily 3. group C. member 1	Nr3c1	nsd	nsd/1.27	nsd/−1.35
1449117_at Jun proto-oncogene related gene d	Jund	nsd	nsd/−1.11	nsd/−1.16
1415949_at carboxypeptidase E///similar to carboxypeptidase E	Cpe///LOC100046434	1.05	1.00/−1.02	−1.05/−1.05
1426205_at similar to protein phosphatase 1/// protein phosphatase 1. catalytic subunit. beta isoform	LOC100044953///Ppp1cb	1.11	nsd/−1.07	nsd/−1.03
1428386_at acyl-CoA synthetase long-chain family member 3	Acsl3	1.32	nsd/−1.26	nsd/nsd
1415964_at stearoyl-Coenzyme A desaturase 1	Scd1	nsd	−1.09/nsd	1.12/1.06
1434377_x_at predicted gene 13654///ribosomal protein S6	Gm13654///Rps6	nsd	1.04/1.02	−1.06/−1.06
1450149_a_at protein phosphatase 1. catalytic subunit. gamma isoform	Ppp1 cc	nsd	nsd/nsd	−1.05/1.05
1428386_at acyl-CoA synthetase long-chain family member 3	Acsl3	1.32	nsd/−1.26	nsd/nsd
Development leptin signaling via PI3 K-dependent pathway/inflammation JAK-STAT pathway				
1443798_at phosphatidylinositol 3-kinase catalytic delta polypeptide	Pik3 cd	1.00	−1.16/−1.06	−1.00/−1.03
1447720_x_at protein kinase. cAMP dependent. catalytic. Alpha	Prkaca	1.03	nsd/nsd	nsd/nsd
1420611_at protein kinase. cAMP dependent. catalytic. Beta	Prkacb	1.08	1.07/nsd	−1.15/−1.10
1452032_at protein kinase. cAMP dependent regulatory. type I. alpha	Prkar1a	1.07	1.05/nsd	nsd/nsd
1434325_x_at protein kinase. cAMP dependent regulatory. type I beta	Prkar1b	−1.03	−1.02/nsd	nsd/nsd
1435638_at glycogen synthase kinase 3 alpha	Gsk3a	nsd	−1.27/nsd	nsd/nsd
1437001_at glycogen synthase kinase 3 beta	Gsk3b	Nsd	nsd/nsd	−1.29/−1.22

Table 7 continued

ID and gene name	Gene symbol	Fold change		
		Protein-restriction R × C	High-fat diet CH × C/RH × R	Taurine CHT × CH/RHT × RH
1420088_at nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor. Alpha	Nfkbia	nsd	nsd/nsd	nsd/−1.16
1415949_at carboxypeptidase E///similar to carboxypeptidase E	Cpe///LOC100046434	1.05	1.00/−1.02	−1.05/−1.05
1455168_a_at guanine nucleotide binding protein (G protein). beta polypeptide 2 like 1	Gnb2l1	1.08	nsd/nsd	nsd/nsd
1456595_x_at growth hormone	Gh	1.05	−459.55/−155.12	nsd/nsd
1429287_a_at Prolactin	Prl	nsd	nsd/−261.44	nsd/nsd

Fold change above zero represents gene up-regulation, and fold change below zero represents gene down-regulation between the two compared groups

$n = 5$ chips per group. pfp <0.1

nsd not significantly different

Fig. 2 Orexigenic and anorexigenic neuropeptide gene expression in the hypothalamus of mice that underwent protein-restriction with or without HFD and Tau supplementation. Hypothalamic mRNA extracts were processed for real time RT-PCR measurement of **a** NPY, **b** AgRP, **c** Pomc and **d** CART gene expression in the C, CH, CHT, R, RH, and RHT groups. Data are mean ± SEM ($n = 5$–8). *Asterisk* different from *C*; *hash* different from *R*. One-way ANOVA followed by Duncan's post-test ($P < 0.05$)
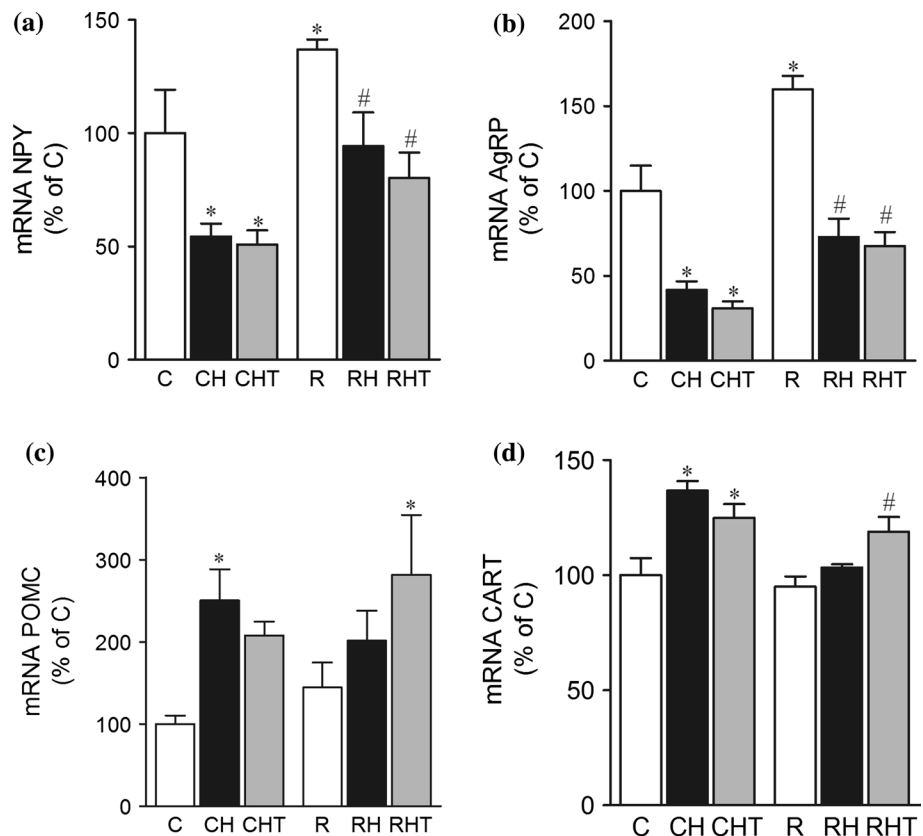
administration significantly reduced the 12 h food intake in the R and C mice ($P < 0.03$ and $P < 0.004$; Fig. 3a). This response was abolished in normal and protein-restricted mice fed a HFD. The anorexigenic response to leptin was restored in both Tau-supplemented groups ($P < 0.04$ in CHT and $P < 0.03$ in RHT; Fig. 3a). In the same mice, the effect of leptin on the hypothalamus was confirmed by the ratio of phosphorylated STAT3 (pSTAT3) to STAT3 total protein after an acute ip leptin administration (Fig. 3b). Leptin induced a 79 and 56 % increase in the pSTAT3/STAT3 protein expression in the C and R groups, respectively, ($P < 0.008$ and $P < 0.04$; Fig. 3b). No modification in pSTAT3/STAT3 content was observed for the RH and CH mice after leptin administration. Tau supplementation

Fig. 3 Food intake profile and hypothalamic STAT3 activation in ▶ response to leptin in mice that underwent protein-restriction with or without HFD and Tau supplementation. **a** Mean ± SEM (n = 4–6) of caloric intake after saline or leptin administration. After a 6 h period of fasting, mice of all groups received an i.p. injection of saline (0.9 % NaCl), and food intake was measured after 12 h. On another day, the same mice received an i.p. injection of leptin (500 ng/g BW). **b** pSTAT3/STAT3 protein expression in the hypothalamus of the C, CH, CHT, R, RH and RHT mice. Mice of both experimental groups received an i.p. injection of 0.9 % saline (−) or leptin (500 ng/g BW) (+). After 45 min, the hypothalamus was collected and used for immunoblotting experiments. **c** STAT3 and GAPDH (internal control) hypothalamic protein content. *Bars* represent the mean ± SEM of the values, determined by optical densitometry (n = 4–6 rats). *Asterisk* leptin is different from saline-treated mice (Student's t test; $P < 0.05$)

increased pSTAT3/STAT3 hypothalamic protein content in the CHT and RHT mice by 69 and 81 %, respectively ($P < 0.02$ and $P < 0.002$; Fig. 3b). No alteration in hypothalamic STAT3 protein expression was observed in the groups (Fig. 3c).

Discussion

Protein-restricted mice showed lower BW, body length, and total protein levels and a higher brain to BW ratio (Tables 1 and 2), which are typical features of a low-protein diet treatment (Amaral et al. 2010; Batista et al. 2012, 2013b). The HFD treatment effectively induced obesity in normal and restricted-protein mice by increasing BW, fat stores, and the plasma levels of CHOL and TG. In accordance with a previous report (Batista et al. 2013b), we also verified that Tau supplementation only prevented fat deposition in normal-protein HFD mice (Table 1).

Protein deficiency during early life may lead to obesity and glucose intolerance in adulthood (Hales and Barker 1992). Recently, our laboratory demonstrated that protein-restricted mice that are fed on a HFD developed obesity, hyperleptinemia, glucose intolerance, and insulin resistance. These features were associated with increased calorie intake (Batista et al. 2013b). Here, using the same rodent model, we aimed to extend these findings through a comprehensive evaluation of food intake, gene expression profile, and central leptin signaling in the hypothalamus. In addition, we investigated whether Tau supplementation modulated these processes, since central administration of this amino acid exerts anorexigenic effects (Solon et al. 2012).

Mice that were fed on a low-protein isocaloric diet showed increased food consumption, yet calorie intake was matched to normal-protein mice (Fig. 1e). This effect was associated with an increase in the expression of NPY and AgRP (Fig. 2; Table 4). In fact, a low-protein diet was reported to result in reduced activation of satiety centers,
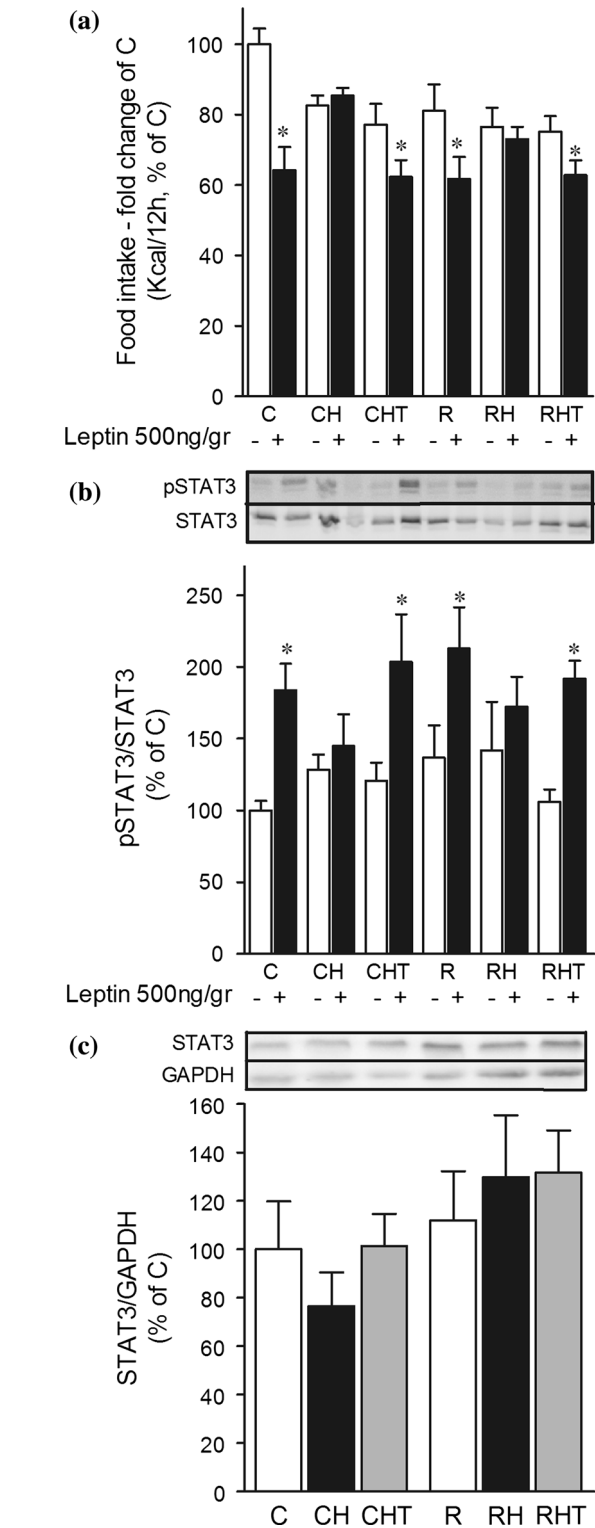
leading to increased food intake (Du et al. 2000). In the off-spring of dams submitted to a protein-restricted diet, during gestation and lactation, hyperphagia associated with an increased expression of NPY and AgRP in the hypothalamus was described (De Souza et al. 2008; Orozco-Solis

et al. 2011). In peripheral tissues, offspring of dams fed on a low-protein diet during gestation showed increased insulin sensitivity at 3 months of age (Latorraca et al. 1998); however, at 17 months, insulin resistance and diabetes were established (Petry et al. 2001). In addition, rats that underwent protein-restriction during lactation showed a lower hypothalamic STAT3 protein expression (Lisboa et al. 2012) and reduced leptin-induced anorexigenic effects (Passos et al. 2004). In contrast to this study, the adaptive gene expression of hypothalamic neuropeptides during protein restriction, observed in our study, was not due to impairment of hypothalamic leptin action, as leptin administration efficiently suppressed food consumption and increased pSTAT3/STAT3 protein expression in the R mice (Fig. 3a, b). Circulating leptin and its gene expression have been consistently reported to be unaltered in mice exposed to protein restriction during gestation or post-weaning (Bol et al. 2009; Batista et al. 2013b). However, we previously demonstrated that R mice had lower pIRS (phosphorylated insulin receptor substrate)-1 protein content in the hypothalamus (Camargo et al. 2013), which may contribute to central insulin resistance and increased orexigenic neuropeptide expression.

In a previous report (Batista et al. 2013b) and in this study, hypophagia was observed during the experimental period in both RH and CH mice, whereas increased caloric intake was verified during the last week of HFD treatment. It is known that HFD modifies hypothalamic neuropeptide expressions during the experimental period. Increased NPY mRNA content in the ARC was observed during the first 2 weeks in diet-induced obesity in rats, whereas at 12 weeks, these rats showed a decrease in NPY gene expression (Levin and Dunn-Meynell 2002). Here, we also observed a reduction in the expression of NPY and AgRP in the hypothalamus of RH and CH mice by microarray and PCR analysis (Fig. 2a, b). Levin and Dunn-Meynell (2002) suggested that an increase in orexigenic neuropeptides may contribute to obesity onset; however, the adaptive reduction of this neuropeptide with the prolongation of high energy intake may act to combat weight gain. Here, we observed increased mRNA content of CART and Pomc in the hypothalamus of normal-protein obese mice, whereas this response was blunted in protein-restricted mice fed on a HFD (Fig. 2c, d). A similar neuropeptide expression profile was previously observed in normal-protein rodents fed on a HFD (Wang et al. 1999; Ziotopoulou et al. 2000). Thus, the protein-restriction background may lead to differential adaptive responses to the HFD.

We also evidenced that the impairment in hypothalamic leptin signaling may be partially involved in caloric food intake and hypothalamic gene expression alterations in the RH and CH mice, since these groups of mice did not present any increase in pSTAT3/STAT3 protein expression

(Fig. 3b) or inhibition of food intake (Fig. 3a) after leptin administration. Hyperleptinemia and impaired response to exogenous leptin are hallmarks of leptin resistance that are frequently described in HFD rodents (Münzberg et al. 2004; Moraes et al. 2009; Koch et al. 2014). Accordingly, leptin was elevated by thirteen- and eightfold in the plasma of CH and RH mice, respectively (Batista et al. 2013b). Taken together our data indicate the development of a full leptin resistance phenotype in both CH and RH mice.

A HFD was shown to increase the expression of inflammatory markers in the hypothalamus (De Souza et al. 2005). This pathway was involved in the desensitization of the hypothalamic leptin action (Romanatto et al. 2007; Amaral et al. 2006). In addition, it is important to mention that, in the hypothalamus of RH and CH mice, a decrease in pIRS-1/IRS-1 protein expression was shown (Camargo et al. 2013), which probably contributed to alterations in hypothalamic gene expressions and feeding behavior in these groups.

In a previous study, Tau showed anorexigenic actions in the hypothalamus by decreasing NPY gene expression and enhancing insulin action (Solon et al. 2012). Here, Tau supplementation had no effect upon the HFD-induced impairment of orexigenic neuropeptides (Table 4; Fig. 2). However, in RHT mice, the mRNA levels of the anorexigenic neuropeptides Pomc and AgRP were elevated (Fig. 2c, d). Furthermore, Tau reduced calorie consumption only in the CHT group (Fig. 1e), while it efficiently preserved the leptin hypothalamic action in both RHT and CHT mice, since both groups showed decreased food intake (Fig. 3a) and increased pSTAT3/STAT3 (Fig. 3b) ratio after leptin injection. Consistent with improved leptin signaling, the HFD-induced hyperleptinemia was previously shown to be completely prevented in CHT but, only partially in RHT mice (Batista et al. 2013b). Solon et al. (2012) reported that intracerebroventricular administration of Tau increased JAK2/STAT3 pathway activation. This amino acid also shows anti-inflammatory properties by decreasing cytokine levels in the brain and peripheral tissues (Lin et al. 2013; Li et al. 2013; Su et al. 2014). In addition, hypothalamic pAkt/Akt protein content was higher in CHT mice (Camargo et al. 2013). This evidence suggests that the preservation of leptin signaling in the hypothalamus partially controls food intake in RHT. As such, since Tau did not improve hypothalamic pAkt expression during basal conditions in this group (Camargo et al. 2013), this mechanism probably contributes to the persistent alteration in calories consumed by RHT mice.

The microarray analyses also demonstrated that the diet and/or Tau supplementation also alters the expression of mRNA related to TAC, glycolysis/gluconeogenesis, and oxidative phosphorylation (Table 5); unfolded protein response, apoptosis, and ER stress pathway (Table 6); and

leptin and insulin signaling and inflammatory pathways (Table 7).

Our results showed that, in the R hypothalamus, there is a reduction in several genes involved in glycolysis/gluconeogenesis, TAC, and oxidative phosphorylation. Previously, modifications in TCA and mitochondrial oxidative phosphorylation genes were reported by microarray analysis in islets isolated from fetal protein-restricted rats (Reusens et al. 2008). In Zucker-fatty rats, the enhanced protein expression of several mitochondrial subunits of the respiratory chain in the hypothalamus leads to a neural glucose hypersensitivity, which impairs the redox signaling, promoting oxidative stress, and more hypothalamic damage (Colombani et al. 2009). In accordance with our results (Table 6), the HFD groups also had an increase in the expression of genes related to the unfolded protein response and ER stress. This pathway is involved in leptin desensitization in the hypothalamus, which may enhance NPY and AgRP mRNA levels, promoting hyperphagia (Ozcan et al. 2009).

Tau shows antioxidant properties and regulates mitochondrial function. Tau normalized the expression of genes involved in TCA, glycolysis, cellular defense, cellular growth and proliferation in fetal pancreatic islets from protein-restricted dams (Reusens et al. 2008). In PC12 cells, Tau decreased the ER stress activation induced by peroxide (Pan et al. 2010). Tau also reduced superoxide production in the mitochondria of cardiomyocytes by enhancing electron transport chain activity (Jong et al. 2012). Protein-restricted mice supplemented with Tau showed better redox balance and a reduction in ER stress markers such as p-PERK and BIP (Batista et al. 2013a, b; Cappelli et al. 2013). In accordance with all these studies, we herein report that Tau supplementation decreased the fold change in genes related to the unfolded protein response, apoptosis, and the ER stress pathway in the hypothalamus of the RHT and CHT mice (Tables 5, 6), which may contribute to better leptin signaling in these groups. Importantly, gene-profiling data was obtained in whole hypothalamus. Thus, further nuclei-specific analyses should provide more insight into Tau's regulatory actions upon gene expression and feeding behavior. Because BBB cells and neurons express Tau transporter (Borg et al.1976; Huxtable and Lippincott 1982; Lee and Kang 2004), it is plausible that at least part of the effects of Tau supplementation described here occur due to a direct action upon the hypothalamic nuclei. In support of this notion, hypothalamic Tau concentration was dramatically elevated in supplemented mice.

In summary, food intake and expression of orexigenic neuropeptides in the hypothalamus were increased by protein restriction and decreased by the HFD, despite increased calorie intake. Tau supplementation exerted profound effects upon hypothalamic gene expression profile that were associated with the prevention of HFD-induced central leptin resistance. The effects of Tau supplementation upon the CNS should be further investigated and considered for the treatment of obesity.

References

Amaral ME, Barbuio R, Milanski M, Romanatto T, Barbosa HC, Nadruz W, Bertolo MB, Boschero AC, Saad MJ, Franchini KG, Velloso LA (2006) Tumor necrosis factor-alpha activates signal transduction in hypothalamus and modulates the expression of pro-inflammatory proteins and orexigenic/anorexigenic neurotransmitters. J Neurochem 98(1):203–212. doi:10.1111/j.1471-4159.2006.03857.x

Amaral AG, Rafacho A, Machado de Oliveira CA, Batista TM, Ribeiro RA, Latorraca MQ, Boschero AC, Carneiro EM (2010) Leucine supplementation augments insulin secretion in pancreatic islets of malnourished mice. Pancreas 39(6):847–855. doi:10.1097/MPA.0b013e3181d37210

Bachmanov AA, Reed DR, Beauchamp GK, Tordoff MG (2002) Food intake, water intake, and drinking spout side preference of 28 mouse strains. Behav Genet 32(6):435–443

Batista TM, Ribeiro RA, Amaral AG, de Oliveira CA, Boschero AC, Carneiro EM (2012) Taurine supplementation restores glucose and carbachol-induced insulin secretion in islets from low-protein diet rats: involvement of Ach-M3R, Synt 1 and SNAP-25 proteins. J Nutr Biochem 23(3):306–312. doi:10.1016/j.jnutbio.2010.12.012

Batista TM, da Silva PM, Amaral AG, Ribeiro RA, Boschero AC, Carneiro EM (2013a) Taurine supplementation restores insulin secretion and reduces ER stress markers in protein-malnourished mice. Adv Exp Med Biol 776:129–139. doi:10.1007/978-1-4614-6093-0_14

Batista TM, Ribeiro RA, da Silva PM, Camargo RL, Lollo PC, Boschero AC, Carneiro EM (2013b) Taurine supplementation improves liver glucose control in normal protein and malnourished mice fed a high-fat diet. Mol Nutr Food Res 57(3):423–434. doi:10.1002/mnfr.201200345

Bernardis LL, Patterson BD (1968) Correlation between 'Lee index' and carcass fat content in weanling and adult female rats with hypothalamic lesions. J Endocrinol 40(4):527–528

Bidlingmeyer BA, Cohen SA, Tarvin TL, Frost B (1987) A new, rapid, high-sensitivity analysis of amino acids in food type samples. J Assoc Off Anal Chem 70(2):241–247

Bol VV, Delattre AI, Reusens B, Raes M, Remacle C (2009) Forced catch-up growth after fetal protein restriction alters the adipose tissue gene expression program leading to obesity in adult mice.

Am J Physiol Regul Integr Comp Physiol 297(2):R291–R299. doi:10.1152/ajpregu.90497.2008

Borg J, Balcar VJ, Mandel P (1976) High affinity uptake of taurine in neuronal and glial cells. Brain Res 118:514–516

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Camargo RL, Batista TM, Ribeiro RA, Velloso LA, Boschero AC, Carneiro EM (2013) Effects of taurine supplementation upon food intake and central insulin signaling in malnourished mice fed on a high-fat diet. Adv Exp Med Biol 776:93–103. doi:10.1007/978-1-4614-6093-0_10

Cappelli AP, Zoppi CC, Barbosa-Sampaio HC, Costa JM, Protzek AO, Morato PN, Boschero AC, Carneiro EM (2013) Taurine-induced insulin signalling improvement of obese malnourished mice is associated with redox balance and protein phosphatases activity modulation. Liver Int. doi:10.1111/liv.12291

Colombani AL, Carneiro L, Benani A, Galinier A, Jaillard T, Duparc T, Offer G, Lorsignol A, Magnan C, Casteilla L, Penicaud L, Leloup C (2009) Enhanced hypothalamic glucose sensing in obesity: alteration of redox signaling. Diabetes 58(10):2189–2197. doi:10.2337/db09-0110

De Souza CT, Araujo EP, Bordin S, Ashimine R, Zollner RL, Boschero AC, Saad MJ, Velloso LA (2005) Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. Endocrinology 146(10):4192–4199. doi:10.1210/en.2004-1520

De Souza CT, Pereira-da-Silva M, Araujo EP, Morari J, Alvarez-Rojas F, Bordin S, Moreira-Filho DC, Carvalheira JB, Saad MJ, Velloso LA (2008) Distinct subsets of hypothalamic genes are modulated by two different thermogenesis-inducing stimuli. Obesity (Silver Spring) 16(6):1239–1247. doi:10.1038/oby.2008.53

Dhillon SS, Belsham DD (2011) Leptin differentially regulates NPY secretion in hypothalamic cell lines through distinct intracellular signal transduction pathways. Regul Pept 167(2–3):192–200. doi:10.1016/j.regpep.2011.01.005

Du F, Higginbotham DA, White BD (2000) Food intake, energy balance and serum leptin concentrations in rats fed low-protein diets. The Journal of nutrition 130(3):514–521

Elmquist JK, Elias CF, Saper CB (1999) From lesions to leptin: hypothalamic control of food intake and body weight. Neuron 22(2):221–232

Flier JS (2004) Obesity wars: molecular progress confronts an expanding epidemic. Cell 116(2):337–350

Gautier L, Cope L, Bolstad BM, Irizarry RA (2004) affy—analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 20(3):307–315. doi:10.1093/bioinformatics/btg405

Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JY, Zhang J (2004) Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 5(10):R80. doi:10.1186/gb-2004-5-10-r80

Gharibani PM, Modi J, Pan C, Menzie J, Ma Z, Chen PC, Tao R, Prentice H, Wu JY (2013) The mechanism of taurine protection against endoplasmic reticulum stress in an animal stroke model of cerebral artery occlusion and stroke-related conditions in primary neuronal cell culture. Adv Exp Med Biol 776:241–258. doi:10.1007/978-1-4614-6093-0_23

Hales CN, Barker DJ (1992) Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia 35(7):595–601

Hong F, Wittner B, Breitling R, Smith C, Battke F (2013) RankProd: rank product method for identifying differentially expressed genes with application in meta-analysis. vol R package version 2.32.0

Huxtable RJ, Lippincott SE (1982) Relative contribution of diet and biosynthesis to the taurine content of the adult rat. Drug Nutr Interact 1:153–168

Jequier E (2002) Leptin signaling, adiposity, and energy balance. Ann N Y Acad Sci 967:379–388

Jong CJ, Azuma J, Schaffer S (2012) Mechanism underlying the antioxidant activity of taurine: prevention of mitochondrial oxidant production. Amino Acids 42(6):2223–2232. doi:10.1007/s00726-011-0962-7

Kahn SE, Hull RL, Utzschneider KM (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444(7121):840–846. doi:10.1038/nature05482

Koch CE, Lowe C, Pretz D, Steger J, Williams LM, Tups A (2014) High-fat diet induces leptin resistance in leptin-deficient mice. J Neuroendocrinol 26(2):58–67. doi:10.1111/jne.12131

L'Amoreaux WJ, Cuttitta C, Santora A, Blaize JF, Tachjadi J, El Idrissi A (2010) Taurine regulates insulin release from pancreatic beta cell lines. J Biomed Sci 17(Suppl 1):S11. doi:10.1186/1423-0127-17-S1-S11

Latorraca MQ, Reis MA, Carneiro EM, Mello MA, Velloso LA, Saad MJ, Boschero AC (1998) Protein deficiency and nutritional recovery modulate insulin secretion and the early steps of insulin action in rats. J Nutr 128(10):1643–1649

Lee NY, Kang YS (2004) The brain-to-blood efflux transport of taurine and changes in the blood-brain barrier transport system by tumor necrosis factor-alpha. Brain Res 1023(1):141–147

Levin BE, Dunn-Meynell AA (2002) Reduced central leptin sensitivity in rats with diet-induced obesity. Am J Physiol Regul Integr Comp Physiol 283(4):R941–R948. doi:10.1152/ajpregu.00245.2002

Li M, Reynolds CM, Sloboda DM, Gray C, Vickers MH (2013) Effects of taurine supplementation on hepatic markers of inflammation and lipid metabolism in mothers and offspring in the setting of maternal obesity. PLoS One 8(10):e76961. doi:10.1371/journal.pone.0076961

Lin S, Hirai S, Yamaguchi Y, Goto T, Takahashi N, Tani F, Mutoh C, Sakurai T, Murakami S, Yu R, Kawada T (2013) Taurine improves obesity-induced inflammatory responses and modulates the unbalanced phenotype of adipose tissue macrophages. Mol Nutr Food Res 57(12):2155–2165. doi:10.1002/mnfr.201300150

Lisboa PC, Oliveira E, Fagundes AT, Santos-Silva AP, Conceicao EP, Passos MC, Moura EG (2012) Postnatal low protein diet programs leptin signaling in the hypothalamic-pituitary-thyroid axis and pituitary TSH response to leptin in adult male rats. Hormone and metabolic research=Hormon- und Stoffwechselforschung=Hormones et metabolisme 44 (2):114–122. doi:10.1055/s-0031-1299747

Moraes JC, Coope A, Morari J, Cintra DE, Roman EA, Pauli JR, Romanatto T, Carvalheira JB, Oliveira AL, Saad MJ, Velloso LA (2009) High-fat diet induces apoptosis of hypothalamic neurons. PLoS One 4(4):e5045. doi:10.1371/journal.pone.0005045

Munzberg H, Myers MG Jr (2005) Molecular and anatomical determinants of central leptin resistance. Nat Neurosci 8(5):566–570. doi:10.1038/nn1454

Münzberg H, Flier JS, Bjørbaek C (2004) Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. Endocrinology 145(11):4880–4889. doi:10.1210/en.2004-0726

Myers MG, Olson DP (2012) Central nervous system control of metabolism. Nature 491(7424):357–363. doi:10.1038/nature11705

Niswender KD, Baskin DG, Schwartz MW (2004) Insulin and its evolving partnership with leptin in the hypothalamic control of energy homeostasis. TEM 15(8):362–369. doi:10.1016/j.tem.2004.07.009

Orozco-Solis R, Matos RJ, Lopes de Souza S, Grit I, Kaeffer B, Manhaes de Castro R, Bolanos-Jimenez F (2011) Perinatal

nutrient restriction induces long-lasting alterations in the circadian expression pattern of genes regulating food intake and energy metabolism. Int J Obes 35(7):990–1000. doi:10.1038/ijo.2010.223

Ozcan L, Ergin AS, Lu A, Chung J, Sarkar S, Nie D, Myers MG, Ozcan U (2009) Endoplasmic reticulum stress plays a central role in development of leptin resistance. Cell Metab 9(1):35–51. doi:10.1016/j.cmet.2008.12.004

Pan C, Giraldo GS, Prentice H, Wu JY (2010) Taurine protection of PC12 cells against endoplasmic reticulum stress induced by oxidative stress. J Biomed Sci 17(Suppl 1):S17. doi:10.1186/1423-0127-17-S1-S17

Passos MC, Vicente LL, Lisboa PC, de Moura EG (2004) Absence of anorectic effect to acute peripheral leptin treatment in adult rats whose mothers were malnourished during lactation. Hormone and metabolic research=Hormon- und Stoffwechselforschung=Hormones et metabolisme 36 (9):625–629. doi:10.1055/s-2004-825927

Petry CJ, Dorling MW, Pawlak DB, Ozanne SE, Hales CN (2001) Diabetes in old male offspring of rat dams fed a reduced protein diet. Int J Exp Diabetes Res 2(2):139–143

Plum L, Belgardt BF, Bruning JC (2006) Central insulin action in energy and glucose homeostasis. J Clin Investig 116(7):1761–1766. doi:10.1172/JCI29063

Prada PO, Zecchin HG, Gasparetti AL, Torsoni MA, Ueno M, Hirata AE, Corezola do Amaral ME, Hoer NF, Boschero AC, Saad MJ (2005) Western diet modulates insulin signaling, c-Jun N-terminal kinase activity, and insulin receptor substrate-1ser307 phosphorylation in a tissue-specific fashion. Endocrinology 146(3):1576–1587. doi:10.1210/en.2004-0767

Purkayastha S, Cai D (2013) Neuroinflammatory basis of metabolic syndrome. Mol Metab 2(4):356–363. doi:10.1016/j.molmet.2013.09.005

Reeves PG (1997) Components of the AIN-93 diets as improvements in the AIN-76A diet. J Nutr 127(5 Suppl):838S–841S

Remacle C, Dumortier O, Bol V, Goosse K, Romanus P, Theys N, Bouckenooghe T, Reusens B (2007) Intrauterine programming of the endocrine pancreas. Diabetes Obes Metab 9(Suppl 2):196–209. doi:10.1111/j.1463-1326.2007.00790.x

Reusens B, Sparre T, Kalbe L, Bouckenooghe T, Theys N, Kruhøffer M, Orntoft TF, Nerup J, Remacle C (2008) The intrauterine metabolic environment modulates the gene expression pattern in fetal rat islets: prevention by maternal taurine supplementation. Diabetologia 51(5):836–845. doi:10.1007/s00125-008-0956-5

Ribeiro RA, Bonfleur ML, Amaral AG, Vanzela EC, Rocco SA, Boschero AC, Carneiro EM (2009) Taurine supplementation enhances nutrient-induced insulin secretion in pancreatic mice islets. Diabetes Metab Res Rev 25(4):370–379

Ribeiro RA, Vanzela EC, Oliveira CA, Bonfleur ML, Boschero AC, Carneiro EM (2010) Taurine supplementation: involvement of cholinergic/phospholipase C and protein kinase A pathways in potentiation of insulin secretion and Ca2+ handling in mouse pancreatic islets. Br J Nutr 104(8):1148–1155. doi:10.1017/S0007114510001820

Romanatto T, Cesquini M, Amaral ME, Roman EA, Moraes JC, Torsoni MA, Cruz-Neto AP, Velloso LA (2007) TNF-alpha acts in the hypothalamus inhibiting food intake and increasing the respiratory quotient–effects on leptin and insulin signaling pathways. Peptides 28(5):1050–1058. doi:10.1016/j.peptides.2007.03.006

Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M (1998) Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 92(4):573–585

Schwartz MW, Woods SC, Porte D, Seeley RJ, Baskin DG (2000) Central nervous system control of food intake. Nature 404(6778):661–671. doi:10.1038/35007534

Solon CS, Franci D, Ignacio-Souza LM, Romanatto T, Roman EA, Arruda AP, Morari J, Torsoni AS, Carneiro EM, Velloso LA (2012) Taurine enhances the anorexigenic effects of insulin in the hypothalamus of rats. Amino Acids 42(6):2403–2410. doi:10.1007/s00726-011-1045-5

Su Y, Fan W, Ma Z, Wen X, Wang W, Wu Q, Huang H (2014) Taurine improves functional and histological outcomes and reduces inflammation in traumatic brain injury. Neuroscience 266:56–65. doi:10.1016/j.neuroscience.2014.02.006

Team RDC (2011) R: a language and environment for statistical computing. R foundation for statistical computing, Saturday, January 1, 2011 (all day), The R Foundation for Statistical Computing, Vienna

Tsuboyama-Kasaoka N, Shozawa C, Sano K, Kamei Y, Kasaoka S, Hosokawa Y, Ezaki O (2006) Taurine (2-aminoethanesulfonic acid) deficiency creates a vicious circle promoting obesity. Endocrinology 147(7):3276–3284. doi:10.1210/en.2005-1007

Vieira E, Marroqui L, Batista TM, Caballero-Garrido E, Carneiro EM, Boschero AC, Nadal A, Quesada I (2012) The clock gene Rev-erbalpha regulates pancreatic beta-cell function: modulation by leptin and high-fat diet. Endocrinology 153(2):592–601. doi:10.1210/en.2011-1595

Wang ZW, Zhou YT, Kakuma T, Lee Y, Higa M, Kalra SP, Dube MG, Kalra PS, Unger RH (1999) Comparing the hypothalamic and extrahypothalamic actions of endogenous hyperleptinemia. Proc Natl Acad Sci USA 96(18):10373–10378

Zabolotny JM, Kim YB, Welsh LA, Kershaw EE, Neel BG, Kahn BB (2008) Protein-tyrosine phosphatase 1B expression is induced by inflammation in vivo. J Biol Chem 283(21):14230–14241. doi:10.1074/jbc.M800061200

Ziotopoulou M, Mantzoros CS, Hileman SM, Flier JS (2000) Differential expression of hypothalamic neuropeptides in the early phase of diet-induced obesity in mice. Am J Physiol Endocrinol Metab 279(4):E838–E845