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Full-length Article

Downregulation of HIF complex in the hypothalamus exacerbates diet-induced obesity☆

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ABSTRACT

Hypothalamic hypoxia-inducible factor-1 (HIF-1) can regulate whole-body energy homeostasis in response to changes in blood glucose, suggesting that it acts as a sensor for systemic energy stores. Here, we hypothesized that hypothalamic HIF-1 could be affected by diet-induced obesity (DIO).

We used eight-week old, male C57Bl6 mice, fed normal chow diet or with high fat diet for 1, 3, 7, 14 and 28 days. The expression of HIF-1alpha and HIF-1beta was measured by PCR and western blotting and its hypothalamic distribution was evaluated by fluorescence microscopy. Inhibition of HIF-1beta in arcuate nucleus of hypothalamus was performed using stereotaxic injection of shRNA lentiviral particles and animals were grouped under normal chow diet or high fat diet for 14 days.

Using bioinformatics, we show that in humans, the levels of HIF-1 transcripts are directly correlated with those of hypothalamic transcripts for proteins involved in inflammation, regulation of apoptosis, autophagy, and the ubiquitin/proteasome system; furthermore, in rodents, hypothalamic HIF-1 expression is directly correlated with the phenotype of increased energy expenditure. In mice, DIO was accompanied by increased HIF-1 expression. The inhibition of hypothalamic HIF-1 by injection of an shRNA resulted in a further increase in body mass, a decreased basal metabolic rate, increased hypothalamic inflammation, and glucose intolerance.

Thus, hypothalamic HIF-1 is increased during DIO, and its inhibition worsens the obesity-associated metabolic phenotype. Thus, hypothalamic HIF-1 emerges as a target for therapeutic intervention against obesity.

1. Introduction

The consumption of large amounts of dietary fats, particularly saturated fatty acids (SFAs), can impair hypothalamic neuronal circuitries that control whole-body energy homeostasis, leading to obesity (De Souza et al., 2005; Dziedzic et al., 2007; Horvath et al., 2010; Moraes et al., 2009; van de Sande-Lee and Velloso, 2012). At least part of this hypothalamic dysfunction emerges as a consequence of an inflammatory response that is triggered as early as one day after the introduction of a high-fat diet (HFD) and thus precedes systemic inflammatory activity, which is a hallmark of the obese phenotype (De Souza et al., 2005; Morari et al., 2014; Souza et al., 2016; Thaler et al., 2012; Valdearcos et al., 2017; Zhang et al., 2008). Increased pro-inflammatory activity is responsible for hypothalamic resistance to leptin and insulin, leading to the impairment of neuronal pathways that control energy homeostasis (Purkayastha et al., 2011; Zhang et al., 2008) and, in later stages, can induce neuronal apoptosis (Guyenet et al., 2013; Li et al., 2012; Moraes et al., 2009). Studies have shown

Abbreviations: ACTH, Adrenocorticotropic Hormone; ARC, Arcuate Nucleus; AgRP, Agouti-Related Peptide; ARNT, Aryl Hydrocarbon Receptor Nuclear Translocator; BAT, Brow Adipose Tissue; DIO, Diet-induced Obesity; FIH, Factor Inhibiting HIF-1α; GFAP, Glial Fibrillary Acidic Protein; GTT, Glucose Tolerance Test; HDF, High Fat Diet; HIF-1, Hypoxic Inducible Factor 1; NPY, Neuropeptide Y; PGC-1alpha, Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha; POMC, Pro-opiomelanocortin; RER, Respiratory Exchange Ratio; SFAs, Saturated Fatty Acids; T2D, Type 2 Diabetes; WAT, White Adipose Tissue; pVHL, Von Hippel–Lindau Tumor suppressor

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that the anorexigenic POMC neuronal subpopulation of the hypothalamic arcuate nucleus (ARC) is the main target of late-stage diet-induced hypothalamic neuronal apoptosis, which may generate an imbalance in orexigenic/anorexigenic responses, favoring the progression of obesity (Horvath et al., 2010; Souza et al., 2016; Torri et al., 2002).

Hypoxia-inducible factor-1 (HIF-1) is a transcription factor that activates several genes in response to hypoxia or other potentially harmful conditions (Semenza, 2012). In the hypothalamus, HIF was shown to control the expression of POMC, impacting on whole body energy homeostasis (Varela et al., 2017; Zhang et al., 2011). The HIF-1 complex is a heterodimer composed of the α and β subunits. The protein levels of the α subunit are regulated by external stimuli, such as hypoxia and inflammation, while the β subunit, also called aryl hydrocarbon receptor nuclear translocator (ARNT), is constitutively present. In addition to its importance in the response to harmful conditions, the HIF-1 complex is also involved in the regulation of glucose and energy homeostasis by acting in β-cells, liver, muscle, adipose tissue, and the hypothalamus (Semenza, 2011; Zhang et al., 2010a), thus contributing to the development of obesity and type 2 diabetes (T2D) (Girgis et al., 2012; Rahtu-Korpela et al., 2014; Zhang et al., 2010b). Particularly, in the hypothalamus, HIF can be regulated by systemic glucose levels and in turn control POMC expression, which is an important component of the neuronal network that modulates whole-body glucose homeostasis (Varela et al., 2017; Zhang et al., 2011).

Because of its tissue-specific role in the control of energy homeostasis, the HIF-1 complex emerges as a potential target for the treatment of metabolic diseases. Here, we hypothesized that the consumption of a HFD could alter the expression/stabilization of the HIF-1 complex in the hypothalamus. We also hypothesized that the hypothalamic HIF-1 complex could be a modulator of systemic metabolism and also a protective factor against hypothalamic inflammation triggered by dietary SFAs, regulating energy expenditure and systemic metabolism. Our results show that downregulation of HIF-1β expression in the ARC increases hypothalamic inflammation, impairs hepatic metabolic function, and decreases energy expenditure, ultimately leading to increased body mass gain. Thus, our data point to an important role of the HIF-1 complex in the hypothalamic regulation of energy expenditure and peripheral metabolism.

2. Material and methods

2.1. Bioinformatic analysis

Correlation analyses were performed from data on hypothalamic mRNA (INIA Hypothalamus AffyMoGene 1.0 ST (Nov10)) and phenotypes (BXD Published Phenotypes) from families of BXD inbred mice and data on hypothalamic mRNA of human individuals (GTEXv5 Human Brain Hypothalamus RefSeq (Sep15) RPKM log2), as previously published(Andreux et al., 2012), all accessible on GeneNetwork (www.genenetwork.org). Heat maps were created using GENE-E (The Broad Institute, www.broadinstitute.org/cancer/software/GENE-E/). The values used are available in Supplementary Tables 1 and 2.

2.2. Experimental animals

Eight-week old, male C57BL6 mice (University of Campinas Central Breeding Center) were randomly assigned to *ad libitum* chow or HFD for 1, 7, 14, or 28 days; water was also available *ad libitum*. Mice were housed in groups, unless stated otherwise, at 22–24 °C, humidity was maintained between 50 and 60% with a 12-h light/dark cycle. Diet composition is presented in Supplementary Table 3. All experiments were performed in accordance with the guidelines of the Brazilian College for Animal Experimentation and approved by the Institutional Animal Care and Use Committee (CEUA 4125-1). Caloric intake and body mass were measured daily for the first 7 days and weekly thereafter. Energetic efficiency was calculated as body weight gained over caloric intake x 100. For experiments aiming at tissue and blood collection, mice were anesthetized with sodium pentobarbital (50 mg/kg body mass, i.p.). Once anesthesia was assured, mice were weighed, and blood samples were collected with cardiac puncture, for measurement of glucose, leptin, and insulin levels. The calculation of body weight gain was performed from the difference between day 28 (the day of sacrifice) and day 0 (just before the introduction of high-fat diet). In this way the weight gain for control group is during the 28 days, as well as for the all HFD groups. Experiments and tissue extractions were always performed between 8 and 10 a.m.

2.3. Immunohistochemistry

For specimen collection for histological studies, mice were deeply anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) and intracardially perfused with saline solution (0.9% NaCl, p.H 7.4) followed by 4% paraformaldehyde (PFA) in saline solution. Brains were removed and post fixed for 24 h in 4% PFA solution and dehydrated in 20% sucrose in 0.1 M PBS. Brains were sectioned on a cryostat at a thickness of 30 µm, and slices were collected (Bregma anterior-posterior from −0.94 mm to −2.30) and stored in an anti-freezing solution (30% ethylene glycol (v/v), 30% sucrose (m/v) in 0.1 M PBS) until used for free-floating immunohistochemistry. For that, slices were washed twice with 0.1 M PBS, blocked with 0.25% Triton X-100 and 5% bovine serum albumin (BSA) in 0.1 M PBS for 2 h at room temperature. Slices were incubated overnight at 4 °C with the primary antibodies in blocking solution [mouse anti-HIF1α (1:200; Novus, #NB100-115), mouse anti-HIF1β (1:200; Novus, #NB100-124), goat anti-POMC (1:200; Abcam #ab32893), rabbit anti-ACTH (1:1000, National Hormone & Peptide Program), goat anti-AgRP (1:200; Santa Cruz Biotechnology, #sc-18634), rabbit anti-GFAP (1:500; Abcam #ab7260), and rabbit anti-IBA1 (1:200; Wako #019-19747)]. After washing, sections were incubated with fluorophore-labeled secondary antibodies in PBS blocking solution for 2 h at room temperature [donkey anti-mouse FITC (1:500, Abcam), donkey anti-rabbit Alexa 564 (1:500, life technology #a10040), donkey anti-goat Cy3 (1:500, Abcam), and donkey anti-rabbit Cy3 (1:500, Abcam)]. Thereafter, the slices were protected from light; nuclei were labeled with DAPI (Sigma Aldrich #D9542) and then mounted in Prolong Antifade fluorescence medium. Sections were examined with a LEICA TCS SP5 II confocal laser-scanning microscope (Leica Microsystems, Wetzlar, Germany).

2.4. RNA extraction and quantitative real time PCR (qRT-PCR)

Total RNA was extracted from mouse hypothalamus by using TRIzol reagent (Invitrogen) according to the manufacturer's recommendations. The cDNA synthesis was performed using 2.0 µg of total RNA according the manufacturer's instructions (High Capacity cDNA Reverse Transcription Kit, Life Technologies). Each PCR contained 25 ng of reverse-transcribed RNA, 0.25 µl of each specific primer, Taqman Universal master mix (Life Technologies), and RNAse-free water to a 10 µl final volume. Real time PCR analysis of gene expression was carried out in an ABI Prism 7500 sequence detection system (Applied Biosystems). Primers were purchased from Applied Biosystems or Integrated DNA Technologies. For RT-PCR calculation we used delta CT and relative gene expression was normalized to that of GAPDH in all samples. All primers' gene bank numbers are listed in Supplementary Table 4. Real-time data were analyzed using the Sequence Detector System 1.7 (Applied Biosystems).

2.5. Protein extraction and western blotting

Following dissection, whole-hypothalamus and brown adipose tissue were homogenized in RIPA lysis buffer [150 mMNaCl, 50 mMTris, 5 mM EGTA, 1% Triton X-100, 0.5% sodium deoxycholate