Original article

MOLECULAR METABOLISM

# PI3K p110β subunit in leptin receptor expressing cells is required for the acute hypophagia induced by endotoxemia

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## ABSTRACT

**Objective:** Hypophagia and increased energy expenditure under inflammatory conditions, such as that observed after bacterial lipopolysaccharide (LPS) administration, are associated with leptin secretion. The hypophagic effect of leptin depends in part on the activation of PI3K signaling pathway. However, the role of PI3K in the endotoxemia-induced hypophagia has not been determined.

**Methods:** In an attempt to examine the functional contribution of the PI3K pathway in hypophagia and weight loss induced by LPS (100 ug/Kg, ip), we performed a central pharmacological PI3K inhibition (LY294002). Additionally, to gain mechanistic insights on the role of the catalytic PI3K p110α subunit in leptin responsive cells, mice expressing Cre-recombinase driven by the *Lepr* promoter (LepR-Cre) were crossed with mice carrying a loxP-modified p110α allele (*Pi3kca* gene) (LepR$^{\Delta p110\alpha}$). As studies have suggested that the PI3K p110β subunit has a dominant role over p110α in energy homeostasis, we further crossed LepR-Cre mice with loxP-modified p110α and p110β (*Pi3kcb* gene) alleles (LepR$^{\Delta p110\alpha+\beta}$). In order to verify the requirement of leptin in PI3K effects on food intake, we also used leptin-deficient *ob/ob* mice.

**Results:** We found that LPS stimulates PI3K and STAT3 signaling pathways in cells expressing the leptin receptor. Central PI3K inhibition prevented LPS-induced hypophagia and weight loss. Genetic deletion of p110α subunit selectively in LepR cells had no effect on LPS-induced hypophagia and weight loss. However, p110α and p110β double deletion in LepR cells prevented LPS-induced hypophagia and partially reversed the weight loss. Leptin deficiency blunted LPS-induced acute pAKT and pSTAT3 phosphorylation and the acute suppression of food intake.

**Conclusions:** Our studies show that the PI3K p110β subunit in LepR cells is required for acute endotoxemic hypophagia. The data provide promising approaches for PI3K inhibition in preventing low energy balance and cachectic states during inflammatory challenges.



**Keywords** LPS; Metabolism; Leptin; Hypothalamus; Inflammation

## 1. INTRODUCTION

Systemic inflammation triggered by bacterial endotoxin is characterized by increased cytokines, altered energy balance via suppression of food consumption and enhanced thermogenesis, body weight loss and behavioral changes [1–4]. These responses and the resulting undernutrition compromise the recovery of the organism. Experimentally, the innate immune system can be activated by administration of lipopolysaccharide (LPS), a cell wall component derived from Gram-negative bacteria. LPS or cytokine injection increases the gene expression and the circulating levels of the proinflammatory adipokine leptin in rodents [5–7]. Leptin is primarily secreted by the white adipose tissue and acts in the brain to control energy homeostasis. Leptin administration decreases food intake and increases energy expenditure [8,9]. The ability of leptin to reduce food intake requires the signal transducer and activation of transcription 3 (STAT3) signaling, which in turn stimulates the transcription of the proopiomelanocortin (*Pomc*) gene, a well-known anorexigenic factor [10,11].

Evidence from several studies indicates that phosphoinositide 3- kinase (PI3K) signaling is an important molecular pathway in metabolic regulation also activated by leptin [12–15]. Leptin triggers PI3K activity via phosphorylation of the insulin receptor substrate-2 (IRS-2) [12,16]. The regulatory subunit p85 then binds to IRS and localizes the catalytic activity to the cell membrane. The PI3K p110 catalytic subunit in turn catalyzes the phosphorylation of PIP2 (phosphatidylinositol 4,5-bisphosphate) to PIP3 (phosphatidylinositol 3,4,5-trisphosphate) that finally recruits and activates downstream molecules [17]. Reports

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Received February 22, 2016 • Revision received March 8, 2016 • Accepted March 14, 2016 • Available online 19 March 2016

http://dx.doi.org/10.1016/j.molmet.2016.03.003

  

using pharmacological approaches have demonstrated that the reduction of food intake by central leptin administration is prevented by pretreatment with PI3K inhibitors [12,13]. The p110α and p110β subunits are critical for the PI3K action on metabolic regulation and are important candidates to mediate leptin's effects [18–20]. Genetic suppression of PI3K activity in hypothalamic neurons blocks the acute effects of leptin on cells activity [21,22].

Luyendyk and coworkers [23] reported that PI3K pathway negatively regulates LPS-induced responses in monocytes and macrophages. However, the role of PI3K in the hypophagia triggered by LPS remains an open question. We hypothesized that leptin-mediated PI3K signaling plays a role in LPS-induced hypophagia. To test this hypothesis, we initially used pharmacologic inhibition of PI3K in mice exposed to endotoxin. In addition, we generated mice lacking either p110α or p110α and p110β isoforms selectively in LepR cells, using the Cre-loxP system. We further assessed the metabolic changes (body weight and food intake) in response to acute LPS treatment and the activation of STAT3 and PI3K pathways. The requirement of leptin in LPS-induced hypophagia, pSTAT3 and pAKT expression was evaluated using leptin-deficient *ob/ob* mice.

## 2. MATERIALS AND METHODS

### 2.1. Ethics statement

All animal procedures were carried out with prior approval from the University of Michigan Committee on Use and Care of Animals (IACUC, Animal Protocol: PRO00004380), in accordance with the guidelines established by the National Institute of Health Guide for the Care and Use of Laboratory Animals, as well as an approval of the Ethics Committee for Animal Use of the School of Medicine of Ribeirao Preto, University of Sao Paulo.

### 2.2. Animals

All animals were kept in a light- (12 h on/off) and temperature- (21–23 °C) controlled environment with free access to water and food. The wild type C57BL/6 (JAX® mice, stock # 000664), the *ob/ob* (JAX® mice, stock # 000632), the LepR-Cre (JAX® mice, stock # 008320), the R26-tdTomato (JAX® mice, stock # 007914), the *Pik3ca* $^{loxP/loxP}$ (JAX® mice, stock # 017704) [24] and the *Pik3cb* $^{loxP/loxP}$ (JAX® mice, stock # 017705) [25] mice were kept in the University of Michigan animal facility. Wild type C57BL/6 mice used for the central injection of the PI3K inhibitor were kept in the Medical School Central Animal Facility of the University of Sao Paulo - Campus of Ribeirao Preto.

In order to visualize the LepRb expressing neurons, we crossed the LepR-Cre, a knock-in strain that coexpresses Cre-recombinase with the *Lepr* gene, previously described and validated [26,27], with the R26-tdTomato mouse, which have a *loxP*-flanked transcription-blocking cassette preventing the expression of CAG promoter-driven tdTomato, a red fluorescent protein. Under Cre-mediated excision of the *loxP*-flanked site, the endogenous red fluorescence is detected only in LepR cells.

### 2.3. LepR-specific deletion of p110α or p110α+β and genotyping

To inactivate the catalytic subunit p110α or both subunits p110α and p110β in LepRb neurons, LepR-Cre mice were crossed with mice carrying the *loxP*-modified p110α (*Pi3kca* gene) and p110β (*Pi3kcb* gene) alleles [24,25]. Preliminary observations indicated that complete Cre-mediated excision is only obtained in LepR-Cre homozygous animals. Therefore, our experimental mice were those homozygous for LepR-Cre allele and homozygous for p110α allele (LepR$^{\Delta p110\alpha}$) or homozygous for pl10α and p110β alleles (LepR$^{\Delta p110\alpha+\beta}$), compared with their respective homozygous littermate controls, p110α$^{loxP}$ and p110α + β$^{loxP}$. Deletion of the p110α and p110β subunits was validated by RT-PCR in arcuate nucleus (ARC) punches from LepR$^{\Delta p110\alpha}$ and LepR$^{\Delta p110\alpha+\beta}$ and their respective control mice. Brains were sliced (thickness: 1.0 mm) according to coordinates from the Franklin and Paxinos mouse brain atlas [28] (−1.3 mm to −2.3 mm from Bregma) and punches of the ARC were microdissected using a stainless-steel punch needle of 1.0 mm in diameter. These mouse lines were also previously used and validated [22].

PCR amplification of the floxed (flanked by *loxP* sites) genomic region, combined with the PCR detection of the Cre transgene in tail-derived DNA, was performed (Sigma RED Extract-N-Amp Tissue PCR Kit -cat# XNAT). Mice were genotyped at weaning and after experiments, using the pairs of primers described in Table 1.

### 2.4. Drugs and animal treatment protocol

Intraperitoneal (ip) injection of saline (0.15M NaCl, in 5 μl/g), LPS (100 μg/kg, in 5 μl/g) from Escherichia coli (Sigma, Serotype 026:B6) or leptin (Sigma, 2.5 μg/g, in 5 μl/g) was performed between 4:00–4:30 PM, 2 h before lights off. The PI3K inhibitor LY294002 (Calbiochem, 1 μg/mouse, in 3 μl) [13] or its vehicle (2% DMSO in 0.15M NaCl, 3 μl) was intracerebroventricularly (icv) injected 30 min before saline or LPS injections. All procedures were performed in 8–10 weeks old male mice.

### 2.5. Experimental procedures

#### 2.5.1. Food intake and body weight phenotyping

Intact mice (n = 6–8/group) were single housed and allowed to adapt to the cages and to handling five days prior to the experiment. On the day of the experiment, food was withdrawn at 04:00 PM and mice received the ip injection of saline or LPS. At 06:00 PM (lights off), the animals were re-fed and food consumption was measured 2, 14 and 24 h afterward. Body weight was determined immediately before the injections and 24 h later. A group of naive wild type mice treated with LPS or saline as described above was subsequently treated with intraperitoneal injection of leptin (2.5 μg/g, ip) or saline for food intake and body weight measurements.

Food intake and body weight were also assessed in wild type mice treated with central injections of the PI3K inhibitor LY294002. For this purpose, eight days before the experiment, anesthetized mice were implanted with a cannula in the lateral ventricle. On the day of the experiment, mice were ascribed to four different groups (n = 8/group): 1) Vehicle + Saline, 2) Vehicle + LPS, 3) LY294002 + Saline, 4) LY294002 + LPS. At 3:30 PM food was withdrawn and mice received an icv injection of vehicle or LY294002. At 04:00 PM, mice received the ip injection of saline or LPS, and 2 h later, the animals were re-fed and food consumption and body weight were measured as described.

#### 2.5.2. Implantation of the cannula into the lateral ventricle

Mice were anesthetized with a mixture of ketamine (60 mg/kg) and xylazine (7.5 mg/kg) at a volume of 0.1 mL/100 g and placed in a stereotaxic instrument (Kopf, model 900). A stainless-steel guide cannula (10 mm) was implanted into the right lateral ventricle (stereotaxic coordinates: AP = −0.3 mm, LL = −1.0 mm and depth = −2.5 mm from the Bregma). The cannula was held in place using two stainless-steel screws and dental acrylic resin in the skull. To prevent occlusion of the guide cannula, a 30 gage metal wire filled the cannula. After surgery, the mice received a prophylactic injection of penicillin (50,000 U, i.p.). Eight days after mice received an icv

 