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Effects of growth hormone on uncoupling protein 1 in white adipose tissues in obese mice

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ABSTRACT

Objective: The transition of white adipocytes to beige cells (a phenomenon referred to as browning or beigeing) during obesity has been previously reported. Our study aimed to examine the mechanisms through which obesity induced by a high fat diet (HFD) affects uncoupling protein 1 (UCP1) expression via signal transduction and activator of transcription 5 (STAT5s).

Design: Seven-week-old male C57BL/6J mice were fed a normal or HFD for 11 weeks. Body weight, white adipose tissue weight, and blood lipid and glucose levels were measured. To unveil the molecular mechanisms of UCP1 expression in adipose tissue, we performed further studying 3T3-L1 cells using qRT-PCR. We also measured UCP1 promoter activity in the TSA201 cell line using a dual luciferase assay. In addition, we analyzed the predicted consensus sequences for STAT5 binding in the UCP1 promoter region.

Results: Mice fed an HFD had higher body weight and intra-abdominal adipose tissues weight and a higher expression of UCP1, GH receptor (GHR), STATs, suppressors of cytokine signaling (SOCSs), and cytokine-inducible SH2-containing protein (CISH) compared to control mice. In 3T3-L1 cell studies, GH induced phosphorylation of the STAT5, SOCSs, CISH and UCP1 expressions. UCP1 promoter activity was associated with constitutively active STAT5 in a dose-dependent manner. We confirmed functional STAT5 binding sites at − 425, − 279, and − 178 bp of the UCP1 promoter.

Conclusion: We suggest that endogenous GH induces UCP1 expression in adipose tissue via STAT5.

1. Introduction

Brown adipose tissue (BAT) contributes significantly to the control of body temperature and energy expenditure in rodents and hibernating animals [1]. BAT has long been thought to be absent or scarce in adult humans; however, recent evidence has proven otherwise [2]. Recent studies suggest that adult humans are capable of developing brown or beige/brite (brown in white) adipocytes, a phenomenon referred to as browning or beigeing, the activation of which might offer novel strategies for the treatment of diverse metabolic diseases, including overweight/obesity [3]. BAT thermogenesis is principally dependent on the β-adrenergically mediated activation of lipolysis and subsequent degradation of fatty acids via uncoupling protein 1 (UCP1), which uncouples mitochondrial oxidative phosphorylation to dissipate the

electrochemical gradient through heat instead of ATP synthesis [4]. Thus, the β-adrenoceptor-UCP1 system presents as an intriguing potential target for the control of energy homeostasis, adiposity, and obesity [5–7].

Mitochondria are the energy source for most eukaryotic cells [8]. Mitochondria perform redox reactions that use the released energy to increase the phosphorylation potential of the cell by phosphorylating ADP into ATP [8]. The oxidation pathway consists of two processes. The metabolic process oxidizes carbon into CO_2 and transmits the reductive power to coenzymes such as NADH and $FADH_2$. Meanwhile, the respiration process re-oxidizes these coenzymes and reduces molecular oxygen to H_2O [8]. The term oxidative phosphorylation reflects this link between oxidation and phosphorylation. On the contrary, the term uncoupling infers the rapid dissipation of energy as heat whenever

substrate and oxygen are available [8]. Brown adipocytes are exceptionally rich in mitochondria that are thought to be naturally uncoupled [8].

UCP1 is a key molecule that is specifically expressed in BAT [4]. β-adrenoceptors (βARs) are critical regulators of lipid metabolism in adipocytes. They are members of the G protein coupled receptor family and respond to catecholamine release from nerve endings. When coupled with the G_s alpha subunit, their activation leads to heightened adenylyl cyclase activity. This, in turn, leads to increased intracellular cAMP levels and induces cAMP-dependent protein kinase A (PKA) activation [9]. A diverse array of thermogenic events are subsequently induced, including lipolysis and UCP1 induction [10]. UCP1 is thought to be solely responsible for adaptive, non-shivering thermogenesis, with no input from either UCP2 or UCP3 [11].

Extensive studies have identified the critical transcriptional regulators involved in thermogenic brown or beige adipocyte differentiation as important therapeutic targets for the reduction of body weight [12–17]. Thus, it follows that it is also of great interest to identify regulatory factors that govern the differentiation program of BAT. However, the critical signaling molecules that precisely control this system are largely unknown. The mitogen-activated protein kinase (MAPK) cascades are some of the major intracellular signaling pathways that are evolutionarily conserved in eukaryotic cells. MAPK pathways regulate multiple cellular responses, including gene induction, cell death, and lipid homoeostasis [12]. In particular, the UCP1 gene expressed in BAT is regulated by the MKK6-p38a pathway [13–15]. Furthermore, estrogen is known to regulate UCP1 expression and is also associated with energy expenditure [16]. PKA-dependent IRE1α-XBP1 activation is also crucial for the transcriptional induction of UCP1 in BAT and represents a novel branch of transcriptional regulation of UCP1 in brown adipocytes [17].

Beige adipocytes arise in white adipose tissue (WAT) in response to various stimuli such as cold exposure, exercise, and diet [18]. Beige adipocytes, unlike white adipocytes, have several small fat droplets and a greater mitochondrial density [18]. Beige adipocytes as well as BAT express the thermogenic protein of UCP1 which uncouples respiration from energy production [18]. Current studies are seeking to determine methods of increasing beige adipogenesis (beigeing/browning). To date, UCP1 has been established as a molecular marker of beigeing and used to study the induction of beige adipogenesis in WAT [19,20].

So far, much effort has been expended defining the transcriptional regulators, reagents, and molecules involved in the induction of beige adipocytes within WAT. As for transcriptional regulators, PR domain containing 16 (PRDM16), peroxisome proliferator-activated receptor-γ (PPARγ), PPARγ coactivator 1α (PGC1α), and CCAAT/enhancer-binding proteinα, as well as various secreted mediators such as bone morphogenetic protein, irisin, fibroblast growth factor, and atrial and brain natriuretic peptides, have been shown to promote the formation of brown-like adipocytes [21]. Additionally, certain pharmacological and nutritional agents can also be involved in promoting WAT browning through the activation of relevant transcription factors or related regulatory signaling pathways [21].

GH is also known to be a regulator of WAT browning [4]. Hioki et al. have reported that GH treatment of the KK-Ay obese mouse model influences the expression of UCP1 mRNA. After treatment with 3.5 mg of GH for 10 days, UCP1 mRNA levels in the KK-Ay obese mice increased 2.8-fold in the BAT and 6.0-fold in the subcutaneous WAT, with no changes seen in the lean control mice. It is very interesting that GH may contribute either directly or indirectly to the reduction of WAT in obese mice only, through modulating UCP1 expression in both BAT and WAT. However, the exact signal cascades that regulate these phenomena are poorly understood. To our knowledge, no detailed mechanisms describing how GH induces the formation of brown-like adipocytes have been reported.

In previous experiments, we also detected UCP1 mRNA expression in the abdominal and epididymal adipose tissue via quantitative real-time polymerase chain reaction (qRT-PCR) [22]. The level of UCP1 expression in the abdominal adipose tissues of HFD mice was significantly higher than seen in similar tissues of mice fed a normal diet (NFD). On the contrary, there was no significant difference between HFD and NFD mice in the expression level of UCP1 in the perirenal, epididymal, or subcutaneous adipose tissues. UCP1 mRNA levels seem to be inconstant, possibly depending on the number of browning cells per volume of fat. Although HFD treatment modified UCP1 mRNA expression levels in every WAT examined, abdominal fat tissue constantly had increased UCP1 mRNA expression, possibly due to an inherent resistance to diet-induced obesity. So, we ask whether UCP1 expression in adipocytes is directly induced by GH via signal transducer and activator of transcription (STAT) family member 5. In this study, we examined the signal transduction pathways activated in obese mice consuming a high-fat diet and assessed possible consensus binding sequences for STAT5 in the UCP1 promoter.

2. Materials and methods

2.1. Animal and treatment

Seven-week-old male C57BL/6J mice were purchased from SLC (Japan SLC, Shizuoka, Japan) and were acclimated in animal facilities under a 12-h light/dark cycle prior to the experiment for two weeks. For eleven weeks, the mice were fed a control, normal-fat diet (NFD: 10% kcal from fat (lard), D12450B, Research Diets Inc., NJ, US) or a high-fat diet (HFD: 45% kcal from fat (lard), D12451, Research Diets Inc., NJ, US). We used HFD feeding as previously described [22]. The HFD group and NFD group consisted of 10 and 8 mice, respectively. Each mouse was fed in a separate cage. Body weight was measured every day and food intake was calculated per cage. Mice were fasted overnight before sacrificing, at which point they were perfused with normal saline from the left ventricle of the heart and the blood, liver, mesenteric adipose, kidney adipose, epididymal adipose, and subcutaneous adipose tissues were collected, weighed, and analyzed.

2.2. Blood serum analysis

Blood samples were placed on ice for 1 h and then centrifuged at $3500 \times g$ for 90 s. The serum was collected and the Oriental Yeast Co., Ltd. (Tokyo, Japan) was commissioned to measure the levels of sugar, total cholesterol (T-CHO), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL−C), and triglycerides (TG).

2.3. Ethical approval

All animal care and handling was performed in accordance with the recommendations in the Guidelines for the Care and Use of Laboratory Animals at Mukogawa Women's University, which fulfills the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). The animal experiment protocols were approved by the Institutional Animal Care and Use Committee at Mukogawa Women's University (Approval No: P-15-2016-01-P).

2.4. Plasmid construction

We analyzed the promoter region of human UCP1 by constructing various deletion mutants. PCR was performed using human genomic DNA as the template. Primers were constructed to include *Xho*I and *Bgl*II restriction enzyme recognition site overhangs on the sense and antisense primers, respectively. The 4254-bp PCR products were subcloned between the XhoI and *Hin*dIII sites of the pGL4.10 vector (Promega, Madison, WI), containing the luciferase gene (luc), resulting in a eukaryotic expression luciferase plasmid, which we named UCP1pro-Luc. The generated plasmids were verified by sequencing. In