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Intestinal epithelial cells promote secretion of leptin and adiponectin in adipocytes

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ABSTRACT

Although leptin and adiponectin are the predominant adipokines, how their circulating levels are regulated is incompletely understood. The present study tested whether intestinal epithelial cells influence the expression and secretion of these adipokines by adipocytes. Leptin gene expression and secretion by cultured human primary adipocytes and Simpson–Golabi–Behmel Syndrome adipocytes increased upon coculture with human enterocytic Caco-2 cells or incubation in conditioned medium of Caco-2 cells. Although adiponectin secretion increased, its mRNA levels decreased. Tissue homogenate of the ileum (but not the jejunum, colon, or liver) of nonobese C57BL/6J mice also stimulated leptin and adiponectin secretion by cultured murine 3T3-L1 adipocytes. However, ileal homogenate of obese KK-Ay mice had no effect on leptin and adiponectin secretion. We propose that as yet unidentified humoral factors released from intestinal epithelial cells are involved in regulating circulating leptin and adiponectin levels. Decreased production of such factors may contribute to hyperphagia in KK-Ay mice.

1. Introduction

White adipose tissue is an endocrine organ that secretes a wide range of protein signals known as adipokines. Leptin, which functions primarily in inhibiting the development of obesity, is a prototypic adipokine that acts through receptors on specific populations of neurons in the brain [1]. The most important factors influencing leptin expression and secretion are the distribution of fat depots and the status of its energy stores because leptin is predominantly expressed in adipocytes, and indeed, circulating leptin concentrations in the fed state are highly correlated with the degree of adiposity [2–4]. In addition, leptin expression and secretion are reportedly promoted by insulin [5–9], glucocorticoids [9,10] and glucose and/or its metabolites [11,12]. Furthermore, infections and inflammation are thought to promote leptin expression and secretion [13–17]. Adiponectin is another major adipokine that is exclusively expressed in adipocytes; in contrast with leptin, circulating adiponectin levels are decreased in obesity [18]. Although much is known about leptin and adiponectin, how their circulating levels are regulated is incompletely understood.

Barbier et al. [19] reported that plasma leptin concentrations increase in rats with trinitrobenzene sulfonic acid–induced colitis or indomethacin-induced ulcerative ileitis, suggesting that mediators released from inflamed intestinal tissue promote leptin expression and secretion by adipocytes. Indeed, leptin expression and secretion are reportedly stimulated by intestinal inflammation–associated inflammatory cytokines such as interleukin (IL)-1α, IL-1β, IL-6 and tumor necrosis factor (TNF)-α [13–17]. However, it is unclear whether leptin expression and secretion are regulated by the intestine under physiologic conditions. Previous studies demonstrated that circulating leptin levels decline after fasting and increase after refeeding [20–23]. The evidence led us to conclude that normal intestinal tissue also influences leptin expression and secretion by adipocytes, although it is conceivable that diet-derived substances such as glucose could stimulate leptin expression and secretion by these cells. Thus, we hypothesized that intestinal epithelial cells regulate adipokine expression and secretion by adipocytes via various as yet unidentified humoral factors. The present study tested this hypothesis using cell culture experiments.

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2. Materials and methods

2.1. Cell culture

Caco-2 human colon carcinoma cells and 3T3-L1 murine preadipocytes were obtained from the American Type Culture Collection. Simpson–Golabi–Behmel Syndrome (SGBS) human preadipocytes were provided by the Division of Pediatric Endocrinology and Diabetes, Department of Pediatrics and Adolescent Medicine, University of Ulm [24]. Caco-2 cells were maintained as previously described [25]. To induce enterocytic differentiation, subconfluent Caco-2 cells were plated onto filter inserts (BD Falcon) in 6-well plastic plates at an initial density of 0.5×10^6 cells/well. Upon reaching confluence (day 0), the cells were cultured for up to 21 days. The transepithelial electrical resistance (TEER) of the Caco-2 monolayer was measured as a marker of enterocytic differentiation [26] using a Millicell-ERS (Merck Millipore). Culture and differentiation of 3T3-L1 and SGBS preadipocytes into adipocytes were performed according to Frost & Lane [27] and Fischer-Posovszky et al. [28], respectively. Culture and differentiation of primary human visceral preadipocytes (poietics human visceral preadipocytes, Lonza) into adipocytes were performed according to the supplier's instructions. 3T3-L1 cells, SGBS cells and primary human visceral preadipocytes were used as differentiated adipocytes 12, 20, and 10 days after induction of differentiation, respectively. Adipocytic differentiation was confirmed by monitoring cells for lipid droplet formation using phase-contrast microscopy (CKX41N-31PHP, Olympus, data not shown).

2.2. Animals and sampling

All study protocols were approved by the Animal Use Committee of Hokkaido University (approval no. 08-0139). Animals were maintained in accordance with the Hokkaido University guidelines for the care and use of laboratory animals. Male C57BL/6J JmsSlc (B6, age 5 weeks) and KK-Ay/TaJcl (KK, age 10 weeks) mice were purchased from Japan SLC and CLEA Japan, respectively. Mice were housed in standard plastic cages in a temperature-controlled (23 ± 2 °C) room under a 12-h light/dark cycle and were allowed free access to water and standard chow diet (MR stock, Nosan Corporation) for 2 weeks. On the last day of feeding, mice were euthanized by exsanguination via severing of the carotid artery under sevoflurane anesthesia. A laparotomy was made and the liver, small intestine, and colon were excised. The proximal and distal halves of the small intestine were defined as the jejunum and ileum, respectively. The luminal contents were thoroughly washed out with ice-cold saline, the jejunum, ileum, and colon were opened longitudinally, and the mucosa was scraped off using a glass slide. Samples of liver and intestinal mucosa were homogenized in ice-cold serum-free DMEM supplemented with penicillin G potassium (100 U/mL), streptomycin sulfate (100 μg/mL) and gentamicin sulfate (50 μg/mL). The homogenates were centrifuged at $14{,}000 \times g$ at 4 °C for 5 min, and the supernatants were filtered using a 0.2-μm syringe filter (Acrodisc). The protein concentration of each sample was measured using a BCA protein assay kit (Thermo Scientific) according to the manufacturer's instructions. All samples were then stored at −80 °C until use.

2.3. Experimental design

For coculture of adipocytes with intestinal epithelial cells, filter inserts on which Caco-2 cells were cultured were placed in the wells of 6-well plates in which adipocytes were cultured (Supplementary Fig. 1A). Both Caco-2 cells and adipocytes were cultured in serum-free RPMI 1640 medium during the pre-coculture and coculture periods (10 and 24 h, respectively). After coculture, the medium was removed from the basal compartment of the filter inserts and subjected to adipokine determination as described below. RNA was isolated from the cells as described below. Adipocytes that were not cocultured (i.e., monocultures) were cultured in serum-free medium for the same amount of time as the pre-coculture and coculture periods. To culture adipocytes in conditioned medium (CM) of Caco-2 cells, CM was removed from the basolateral compartment of filter inserts on which Caco-2 cells had been cultured in serum-free RPMI 1640 medium for 24 h (Supplementary Fig. 1B). Adipocytes were cultured with serum-free medium supplemented with different concentrations of CM for 0, 15, 24 or 48 h, after which the medium was subjected to adipokine determination as described below. Cells were subjected to determination of total triglycerides (TGs) as described below. Unless otherwise noted, Caco-2 cells at least 14 days after reaching confluence were used for coculture and CM experiments. 3T3-L1 adipocytes were also cultured for 24 h in serum-free DMEM supplemented with the filtrates of murine tissue homogenates prepared as described above (Supplementary Fig. 1C).

2.4. ELISA

The concentrations of adiponectin, leptin and resistin in the culture medium were determined using ELISA kits (Adiponectin/Acrp30 DuoSet, Leptin DuoSet ELISA Development and Resistin Quantikine ELISA kit, R&D Systems) according to the manufacturers' instructions.

2.5. Colorimetric determination of TGs in adipocytes

After removal of the medium, SGBS cells were lysed by sonication in a buffer composed of 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.1% (w/v) sodium dodecyl sulfate, 1% (w/v) Triton X-100 and 1% (w/v) sodium deoxycholate. The concentration of TGs in the lysates was measured using a Triglyceride E-Test (Wako Pure Chemical Industries) according to the manufacturer's instructions.

2.6. Analysis of mRNA expression

Total RNA was isolated from adipocytes using a ReliaPrep RNA Cell Miniprep System (Promega) and reverse transcribed to generate first-strand cDNA using a ReverTra Ace qPCR RT kit (Toyobo) according to the manufacturer's instructions. To compare the steady-state levels of mRNAs encoding adipocytokines and adipocytic differentiation-related proteins, real-time quantitative PCR (RT-qPCR) was performed using GeneAce SYBR qPCR Mix α No ROX (Nippon Gene) with a Thermal Cycler Dice TP800 (Takara Bio) according to the manufacturer's instructions. The sequences of primers used for RT-qPCR are shown in Supplementary Table 1. Relative mRNA expression levels for each sample were normalized to that of glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

2.7. Statistical analysis

Results are presented as means and SEM. Unpaired *t*-tests were used to compare means between two groups. Dunnet's or Tukey–Kramer's multiple comparison tests following one-way analysis of variance was used to compare mean values between three or more groups. Data were analyzed using GraphPad Prism for Macintosh (version 6, GraphPad Software). P values <0.05 were considered to indicate statistical significance.