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RESEARCH ARTICLE

Extra-adrenal glucocorticoids contribute to the postprandial increase of circulating leptin in mice

Yuka Tomabechi[1] · **Takeshi Tsuruta**[2] · **Shinichi Saito**[3] · **Martin Wabitsch**[4] · **Kei Sonoyama**[5]

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Abstract Leptin, an adipokine secreted by white adipocytes, is known for its function in regulating food intake and energy expenditure, but the mechanisms regulating its circulating levels is not fully understood. Our previous findings suggest that as yet unidentified humoral factors released from enterocytes are involved. The present study tested glucocorticoids (GCs) as candidate factors. Supplementation of corticosterone and cortisol promoted leptin production in murine adipocytes from the 3T3-L1 cell strain and human adipocytes from the Simpson Golabi–Behmel syndrome (SGBS) cell strain, respectively. These changes were observed in the absence but not presence of the GC-receptor antagonist mifepristone. The cortisol concentration in conditioned medium (CM) of human enterocyte-like Caco-2 cells was increased by phorbol-12-myristate 13-acetate and decreased by metyrapone. When SGBS adipocytes were cultured in these CMs, leptin production was positively associated with cortisol concentrations. During a 2-h refeeding after fasting, plasma leptin levels continued to increase in sham-operated mice, transiently increased at 60 min in adrenalectomized mice, and were unchanged in mifepristone-administered mice. These results suggest that extra-adrenal GCs contribute to the GC-receptor signaling-dependent increase of postprandial circulating leptin, whereas further studies will be required to determine whether enterocytes participate in the GCs-mediated increase of postprandial circulating leptin.

Keywords Leptin · Adipocyte · Glucocorticoid · Enterocyte

Abbreviations

B6	C57BL/6
CM	Conditioned medium
GC	Glucocorticoid
MET	Metyrapone
Mif	Mifepristone
PMA	Phorbol-12-myristate 13-acetate
RT-qPCR	Real-time quantitative PCR
SGBS	Simpson Golabi–Behmel syndrome

Introduction

Leptin, a 16-kDa polypeptide synthesized and secreted mainly by white adipocytes, plays a role in weight control by suppressing food intake and increasing energy expenditure through acting on specific populations of neurons in the brain (Pan et al. 2014). The most important factors influencing leptin expression and secretion are the distribution of fat depots and the status of their energy stores, because leptin is predominantly expressed in adipocytes. In the fed state, circulating leptin concentrations are highly correlated with the degree of adiposity (Frederich et al. 1995; Hamilton et al. 1995; Maffei et al. 1995). However, circulating leptin levels decline after fasting and increase after refeeding (Ahrén et al. 1997; Boden

✉ Kei Sonoyama
ksnym@chem.agr.hokudai.ac.jp

[1] Graduate School of Life Science, Hokkaido University, Sapporo 060-8589, Japan

[2] Graduate School of Environmental and Life Science, Okayama University, Okayama 700-8530, Japan

[3] Graduate School of Medicine, Yamaguchi University, Ube 755-8505, Japan

[4] Department of Pediatrics and Adolescent Medicine, University of Ulm, 89075 Ulm, Germany

[5] Laboratory of Food Biochemistry, Research Faculty of Agriculture, Hokkaido University, Kita-9 Nishi-9, Kita-ku, Sapporo 060-8589, Japan

et al. 1996; Hardie et al. 1996; Weigle et al. 1997), suggesting that circulating leptin levels are also controlled by feeding status in addition to adiposity status. Glucose (Wellhoener et al. 2000) and insulin (Fukuda and Iritani 1999; Saladin et al. 1995) reportedly stimulate the expression and secretion of leptin in adipocytes, whereas circulating free fatty acids serve as suppressors of leptin secretion (Pan et al. 2014). Although these factors may be involved in the regulation of circulating leptin levels in terms of feeding status, the entire picture of the regulation remains to be elucidated.

We recently reported that leptin expression and secretion by cultured adipocytes differentiated from Simpson-Golabi-Behmel Syndrome (SGBS) human preadipocytes are promoted by coculturing with human enterocyte-like Caco-2 cells (Ishihara et al. 2015). In addition, the conditioned medium (CM) of Caco-2 cells also promote leptin secretion by SGBS adipocytes (Ishihara et al. 2015). These observations suggest that humoral factors produced by enterocytes promote leptin expression and secretion by adipocytes. However, the humoral factors remain unidentified. Previous studies have shown that leptin expression and secretion in adipocytes are promoted by glucocorticoids (GCs) in vitro (Murakami et al. 1995; Slieker et al. 1996; Wabitsch et al. 1996) and in vivo (Jahng et al. 2008). In addition, the production of corticosterone has been demonstrated in murine small intestinal mucosa ex vivo, and the gene expression of steroidogenic enzymes has been also shown in the small intestinal crypt cells by *in situ* hybridization (Cima et al. 2004). Furthermore, colon cancer cell lines including Caco-2 cells reportedly produce cortisol (Sidler et al. 2011). Therefore, we hypothesized that GCs are enterocyte-derived humoral factors that promote leptin production in adipocytes. The present study tested this hypothesis. Initially, we examined the effect of GCs supplementation on the leptin production in adipocytes in vitro. Secondly, leptin production was also examined in SGBS adipocytes supplemented with CM of Caco-2 cells cultured with phorbol-12-myristate 13-acetate (PMA) or metyrapone (MET). PMA reportedly promotes gene expression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), a key enzyme that catalyzes the intracellular conversion of inert GCs to physiologically active GCs, in the murine intestinal epithelial cell line mICcl2 (Mueller et al. 2007), while MET has been known to be an inhibitor of cortisol synthesis (Sangild et al. 1995). Thus, we intended to modulate the cortisol production in Caco-2 cells and then to test whether leptin production in SGBS adipocytes was influenced by CM of Caco-2 cells in which the cortisol production was altered. Thirdly, we tested whether extra-adrenal GCs promote circulating leptin in mice. Because the adrenal glands are the major source of endogenous GCs, we employed the adrenalectomized mice and compared their circulating leptin levels with those in GC-receptor antagonist-administered mice.

Materials and methods

Cell culture

Caco-2 human colon carcinoma cells and 3T3-L1 murine preadipocytes were obtained from the American Type Culture Collection (Rockville, MD). SGBS cells were provided by the Division of Pediatric Endocrinology and Diabetes, Department of Pediatrics and Adolescent Medicine, University of Ulm (Wabitsch et al. 2001). Caco-2 cells were maintained and induced to enterocytic differentiation as previously described (Ishihara et al. 2015). Culture and differentiation of 3T3-L1 and SGBS preadipocytes into adipocytes were performed as described previously (Ishihara et al. 2015). 3T3-L1 and SGBS cells on days ten and 15 of induction of differentiation, respectively, were used as differentiated adipocytes. Adipocytic differentiation was confirmed by monitoring cells for lipid droplet formation using phase-contrast microscopy (CKX41N-31PHP, Olympus, Tokyo, Japan, data not shown).

Experimental design

Differentiated 3T3-L1 and SGBS adipocytes were cultured in fetal calf serum-free medium supplemented with different concentrations (0, 10, 100, and 1000 ng/mL) of corticosterone and cortisol, respectively, for 24 h in the presence or absence of the GC receptor antagonist mifepristone (Mif, 1 μM, Sigma-Aldrich Japan, Tokyo, Japan). To culture SGBS adipocytes in CM of Caco-2 cells, CM was obtained from the basolateral compartment of filter inserts on which Caco-2 cells had been cultured in medium supplemented with or without the GC synthesis promoter PMA (3 ng/mL, Wako Pure Chemical Industries, Osaka, Japan) or the GC synthesis inhibitor MET (200 μg/mL, Tocris Bioscience, Ellisville, MO) for 24 h. SGBS adipocytes were cultured in the CM for 24 h in the presence or absence of Mif (1 μM). Culture supernatants of 3T3-L1 and SGBS adipocytes were subjected to leptin ELISA as described below, and 3T3-L1 adipocytes were subjected to RNA isolation as described below. Caco-2 cell CMs were subjected to cortisol ELISA as described below.

Animal experiments

All study protocols were approved by the Animal Use Committee of Hokkaido University (approval no. 14–0028). Animals were maintained in accordance with the Hokkaido University guidelines for the care and use of laboratory animals. Male C57BL/6 J JmsSlc (B6, age 6 weeks) mice were purchased from Japan SLC (Shizuoka, Japan). 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, Yokohama, Japan). After feeding the diet for 1 week, food was deprived for 18 h. Under intraperitoneal ketamine and xylasine (80 and 8 mg/kg body weight, respectively) anesthesia, bilateral adrenalectomies (Adx group, six mice) and sham operations (12 mice) were performed according to Uchoa et al. (2012). Adx mice were given 0.9% saline as drinking water. After recovery for 1 week, six sham-operated mice were orally administered Mif (60 mg/kg bodyweight) in 0.5% carboxymethyl cellulose (Mif group), and Adx mice and the remaining six sham-operated mice (Sham group) were administered the vehicle. After withdrawal of food for 18 h, Mif solution and the vehicle were similarly administered again. All mice were then refed the standard chow diet for 120 min. Blood samples were obtained as described above, and the plasma was subjected to ELISA for leptin and corticosterone measurements as described below.

ELISA

For the measurement of leptin in the culture supernatants of SGBS adipocytes, each well of 96-well microtiter plates was coated with anti-human leptin antibody (MAB398, R&D Systems, Minneapolis, MN) overnight at 4°C. After blocking the wells with 1% bovine serum albumin in phosphate-buffered saline (PBS) overnight at 4°C, samples and standard solutions (recombinant human leptin [398-LP-01M, R&D Systems]) were added and then incubated overnight at 4°C. Biotin-conjugated anti-human leptin antibody (BAM398, R&D Systems) was then added and incubated for 2 h at room temperature. Next, horseradish peroxidase-conjugated streptavidin (R&D Systems) was added and incubated for 30 min at room temperature. Plates were developed at room temperature after the addition of TMB substrate solution (eBioscience, San Diego, CA). After the addition of 2 N H_2SO_4, the absorbance at 450 nm was recorded on a microplate reader (Multi-Mode Microplate Readers, BioTek, Santa Barbara, CA). The wells were washed between each step with 0.05% Tween 20 in PBS. ELISA for the measurement of leptin in the culture supernatants of 3T3-L1 adipocytes and the plasma samples of mice was performed in the same manner except that anti-mouse leptin antibody, biotin-conjugated anti-mouse leptin antibody, and recombinant mouse leptin (AF498, BAF498, and 498-OB-01M, respectively, R&D Systems) were used for the capture antibody, detection antibody, and standard, respectively. The concentrations of cortisol in the culture supernatants of Caco-2 cells were determined using an ELISA kit (ADI-900-071, Enzo Life Sciences, Farmingdale, NY) according to the manufacturer's instructions. The concentrations of corticosterone in the plasma samples of mice were determined using an ELISA kit (K014-H1, Arbor Assays, Ann Arbor, MI) according to the manufacturer's instructions.

Analysis of mRNA expression

Total RNA was isolated from 3T3-L1 adipocytes using a ReliaPrep RNA Cell Miniprep System (Promega Japan, Tokyo, Japan) and reverse transcribed to generate first-strand cDNA using a ReverTra Ace qPCR RT kit (Toyobo, Osaka, Japan) according to the manufacturer's instructions. Murine leptin is encoded by *Lep* gene. To compare the steady-state levels of *Lep* mRNAs, real-time quantitative PCR (RT-qPCR) was performed using GeneAce SYBR qPCR Mix α No ROX (Nippon Gene, Toyama, Japan) with a Thermal Cycler Dice TP800 (Takara Bio, Otsu, Japan) according to the manufacturer's instructions. Relative mRNA expression levels for each sample were normalized to that of *Gapdh*. The sequences of primers used for RT-qPCR were as follows: *Lep*, forward 5′-aagtccaggatgacaccaaaacc-3′ and reverse 5′-ggtccatcttggacaaactcagaa-3′ (Phrakonkham et al. 2008); *Gapdh*, forward 5′-tcaccaccatggagaaggc-3′ and reverse 5′-gctaagcagttggtggtgca-3′ (Zhang et al. 2008).

Statistical analyses

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

Results

GCs promote leptin production by adipocytes in the absence but not presence of a GC receptor antagonist

The concentrations of leptin in culture supernatants of murine 3T3-L1 adipocytes were significantly higher in cells cultured with 1000 ng/mL, but not 10 or 100 ng/mL, corticosterone than in those cultured without corticosterone (Fig. 1a). In contrast, the leptine concentrations were the same regardless of corticosterone supplementation in the presence of the GC receptor antagonist Mif. Likewise, *Lep* mRNA levels were significantly higher in 3T3-L1 adipocytes cultured with 100 and 1000 ng/mL, but not 10 ng/mL, corticosterone than in those cultured without corticosterone, and the levels were the same in the presence of Mif (Fig. 1b). In human SGBS adipocytes, the concentrations of leptin in culture supernatants were significantly higher in cells cultured with 10, 100, and 1000 ng/

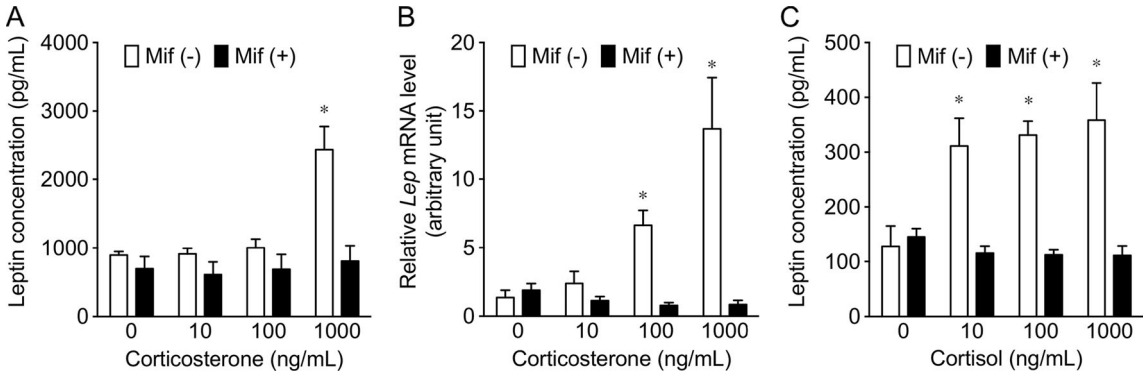

Fig. 1 Effect of glucocorticoids (GCs) on leptin production in adipocytes. **a** leptin concentrations in culture supernatants of 3T3-L1 adipocytes cultured in medium supplemented with different concentrations of corticosterone for 24 h in the presence (*closed columns*) or absence (*open columns*) of mifepristone (Mif, 1 μM). **b** *Lep* mRNA levels in 3T3-L1 adipocytes cultured as described in *A*. **c** leptin concentrations in culture supernatants of SGBS adipocytes cultured in medium supplemented with different concentrations of cortisol for 24 h in the presence (*closed columns*) or absence (*open columns*) of Mif (1 μM). Data are expressed as the means and SEM of three independent experiments. Mean values with asterisks were significantly different ($p < 0.05$) *vs.* values in the absence of GCs as estimated by Dunnett's multiple comparison test following one-way ANOVA

mL cortisol than in those cultured without cortisol, and the concentrations were the same in the presence of Mif (Fig. 1c).

GC concentrations in Caco-2 cell CM are associated with increased leptin from SGBS adipocytes

We cultured Caco-2 cells in the presence of PMA and MET to alter GC production (Fig. 2a). The concentrations of cortisol in culture supernatants of Caco-2 cells were significantly higher and lower in cells cultured with PMA and MET, respectively, as compared to cells cultured without PMA or MET (Fig. 2b). SGBS adipocytes were cultured in these Caco-2 cell CMs (Fig. 2a). In the absence of Mif, the concentrations of leptin in culture supernatants were significantly higher in cells cultured in untreated Caco-2 cell CM than in those cultured in basal medium (Fig. 2c). Furthermore, leptin

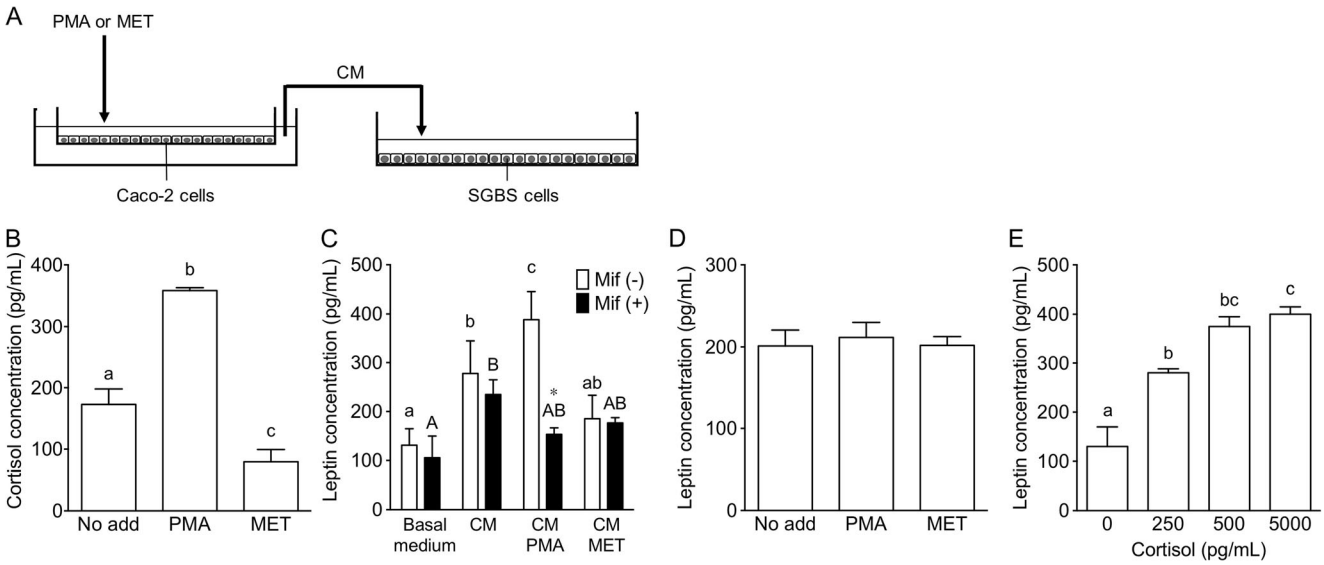

Fig. 2 Effect of conditioned medium (CM) of Caco-2 cells on leptin production in SGBS adipocytes. **a** the experimental design. **b** cortisol concentrations in culture supernatants of Caco-2 cells cultured in medium supplemented with or without phorbol-12-myristate 13-acetate (PMA, 3 ng/mL) or metyrapone (MET, 200 μg/mL) for 24 h. **c** leptin concentrations in culture supernatants of SGBS adipocytes cultured in CM of Caco-2 cells for 24 h in the presence (*closed columns*) or absence (*open columns*) of mifepristone (Mif, 1 μM). **d** leptin concentrations in culture supernatants of SGBS adipocytes cultured in medium supplemented with or without PMA (3 ng/mL) or MET (200 μg/mL) for 24 h. **e** leptin concentrations in culture supernatants of SGBS adipocytes cultured in medium supplemented with different concentrations of cortisol for 24 h. Data are expressed as the means and SEM of three independent experiments. In **b** and **e** mean values not sharing a letter were significantly different ($p < 0.05$) as estimated by Tukey-Kramer's multiple comparison test following one-way ANOVA. In *C*, the value in Mif (+) with an asterisk was significantly different ($p < 0.05$) *vs.* Mif (−) as estimated by unpaired *t*-test, and values not sharing a letter within Mif (−) and Mif (+) were significantly different ($p < 0.05$) as estimated by Tukey-Kramer's multiple comparison test following one-way ANOVA

concentrations were significantly higher in cells cultured in PMA-treated Caco-2 cell CM than in those cultured in untreated Caco-2 cell CM. Leptin concentrations in culture supernatants of cells cultured in MET-treated Caco-2 cell CM did not differ from those in cells cultured in basal medium. In the presence of Mif, leptin concentrations in culture supernatants were significantly higher in cells cultured in untreated Caco-2 cell CM than in those cultured in basal medium (Fig. 2c). In cells cultured in PMA-treated and MET-treated Caco-2 CMs, leptin concentrations were intermediate between cells cultured in untreated Caco-2 cell CM and basal medium. Leptin concentrations in culture supernatants were the same between SGBS adipocytes supplemented with and without PMA and MET (Fig. 2d). Because cortisol concentrations (up to approximately 350 pg/mL, Fig. 2b) in culture supernatants of Caco-2 cells were much lower than those examined for SGBS adipocytes (10, 100, and 1000 ng/mL, Fig. 1c), we re-examined whether such lower concentrations of cortisol could also promote leptin production in SGBS adipocytes. As shown in Fig. 2e, cortisol supplementation with lower concentrations (250, 500, and 5000 pg/mL) significantly increased leptin concentrations in culture supernatants in a dose-dependent manner. No leptin was detected in Caco-2 cell CMs.

Adx and Mif affect postprandial increase of plasma leptin in mice

After 18-h fasting, plasma leptin concentrations were the same between Sham, Adx, and Mif mice (0.67 ± 0.40, 0.48 ± 0.33, and 0.40 ± 0.35 ng/mL, respectively). Figure 3a shows the time-course changes in the plasma leptin levels during refeeding relative to the value at 0 min which was set to 1.0 in each group. The plasma leptin levels in Sham mice continued to increase during refeeding, and the levels were significantly higher at 60 and 120 min than at 0 min. In Adx mice, the plasma leptin levels increased transiently at 60 min. In contrast, no significant difference was observed in the plasma leptin levels of Mif mice during refeeding. The plasma leptin level at 60 min was significantly higher in Sham and Adx mice than in Mif mice, and the level at 120 min was significantly higher in Sham mice than in Adx and Mif mice. Figure 3b showed the time-course changes in the plasma corticosterone concentrations. The concentrations in Sham, Adx, and Mif mice remained unchanged during refeeding, whereas the concentrations were significantly lower in Adx mice than in Sham and Mif mice during all that time. No significant difference was observed in the corticosterone concentrations between Sham and Mif mice.

Discussion

The present study clearly showed that GCs promote leptin production at the pre-translational level in adipocytes in vitro. These results are in line with previous studies in vitro (Murakami et al. 1995; Slieker et al. 1996; Wabitsch et al. 1996) and in vivo (Jahng et al. 2008). We also found that the stimulating effect of GCs on leptin production in adipocytes was abolished by a GC-receptor antagonist. These findings suggest that GCs stimulate leptin production through GC-receptor signaling in adipocytes.

We previously observed that Caco-2 cell CM promotes leptin production in SGBS adipocytes, suggesting that Caco-2 cells secrete some humoral factors that stimulate leptin production in SGBS adipocytes (Ishihara et al. 2015). To test whether GCs are such humoral factors, in the present study we modulated GC production in Caco-2 cells by culturing in

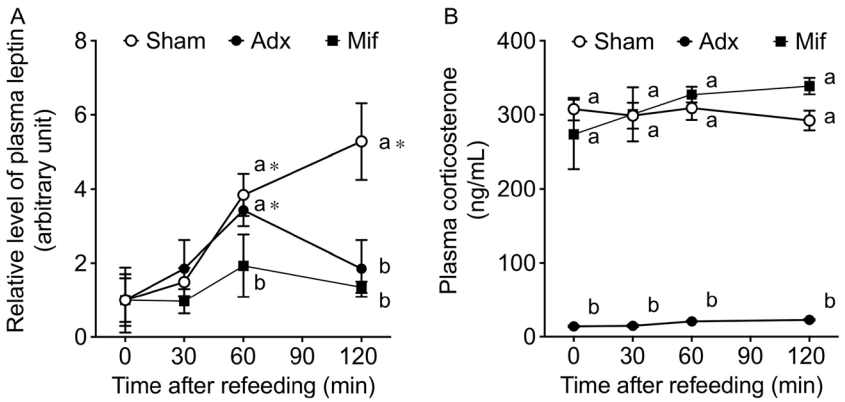

Fig. 3 Time-course changes in the plasma leptin and corticosterone levels (**a** and **b**, respectively) in sham-operated mice (Sham), adrenalectomized mice (Adx), and mifepristone-administered mice (Mif) during refeeding. For the plasma leptin, values at 30, 60, and 120 min are shown relative to the value at 0 min which was set to 1.0 in each group. Data are expressed as the means and SEM of six mice. Mean values with asterisks were significantly different ($p < 0.05$) *vs.* the value at 0 min in each group as estimated by Dunnett's multiple comparison test following one-way ANOVA. Mean values not sharing a letter at each time point were significantly different ($p < 0.05$) as estimated by Tukey-Kramer's multiple comparison test following one-way ANOVA

medium supplemented with the GC-synthesis promoter PMA (Mueller et al. 2007) and inhibitor MET (Sangild et al. 1995). As expected, the concentration of cortisol in the culture supernatant was increased by PMA and decreased by MET. We cultured SGBS adipocytes in the PMA- and MET-treated Caco-2 cell CMs. The stimulating effect of Caco-2 CM on leptin production in SGBS adipocytes was significantly upregulated by PMA treatment and somewhat downregulated by MET treatment. These results indicate that cortisol concentrations in Caco-2 CM are positively associated with leptin concentrations in culture supernatants of SGBS adipocytes. Although the effect of PMA-treated Caco-2 cell CM was abolished by the GC receptor antagonist, we found no influence of the GC receptor antagonist on leptin concentrations in culture supernatants of SGBS adipocytes cultured in untreated CM. From these results, we speculate that additional humoral factors derived from Caco-2 cells might promote leptin production in SGBS adipocytes in a GC receptor-independent manner. We found that leptin concentrations in culture supernatants of SGBS adipocytes were unchanged by supplementation with PMA and MET, suggesting no direct effect of PMA and MET on leptin production. Taken together, the present findings suggest that cortisol derived from Caco-2 cells stimulates leptin production through GC-receptor signaling in SGBS adipocytes.

Previous studies have demonstrated that food consumption promotes circulating leptin levels (Ahrén et al. 1997; Boden et al. 1996; Hardie et al. 1996; Weigle et al. 1997). To test whether GCs mediate the increase of leptin production during refeeding, we compared plasma leptin levels during refeeding in Sham, Adx, and Mif-treated mice. We observed an increase in plasma leptin during refeeding in Sham mice, which is consistent with previous reports (Ahrén et al. 1997; Boden et al. 1996; Hardie et al. 1996; Weigle et al. 1997). In contrast, Mif-treated mice failed to show increased plasma leptin levels upon refeeding, suggesting that GC-receptor signaling plays a definitive role in the upregulation of leptin production in response to food intake. A previous study showed that plasma leptin levels were the same before and after a 2-h refeeding in Adx rats (Uchoa et al. 2012). In the present study, we examined the time-course changes in the plasma leptin levels during a 2 h-refeeding period. We found that Adx mice showed a transient increase of plasma leptin at 60 min. Given that the increase of postprandial circulating leptin depends on GC-receptor signaling, it is conceivable that extra-adrenal GCs might contribute to the increase of circulating leptin, in particular, during early phase of refeeding. Even though the adrenal glands were surgically resected, however, it would be difficult to exclude completely the possibility that the residual adrenal GCs still exist and contribute to the increase of circulating leptin in Adx mice. In addition, even though the present in vitro experiments suggest that enterocyte-derived GCs stimulate leptin production in adipocytes through GC-receptor signaling, it remains unclear whether enterocyte-derived GCs contribute to the increase of circulating leptin during refeeding in vivo.

Because Mif is also known as a progesterone receptor antagonist, one may presume that circulating progesterone is responsible for the increase of circulating leptin during refeeding. Indeed, Stelmanska et al. (2012) showed that progesterone administration to female rats increased leptin mRNA levels in adipose tissues and that Mif treatment abolished the effect of progesterone. In contrast, the authors showed, in adipose tissues of male rats, that increased circulating progesterone failed to increase leptin mRNA levels and that progesterone receptor mRNA was hardly detected (Stelmanska et al. 2012). Because the present study employed male mice, it seems unlikely that observed unresponsiveness of circulating leptin in Mif mice during refeeding is a consequence of inhibition of progesterone receptor signaling.

Although the increase of postprandial circulating leptin might depend on GC-receptor signaling as suggested by the present study, we observed no temporal changes in the plasma corticosterone concentrations during refeeding. One explanation for these results is that local but not systemic GCs promote leptin production in adipocytes during refeeding. 11β-HSD1 is a key enzyme that catalyzes the intracellular conversion of inert GCs to physiologically active GCs in the liver, vasculature, brain, and adipose tissue (Seckl and Walker 2001). We are currently investigating whether 11β-HSD1 in adipose tissue is involved in the postprandial increase of leptin production.

In addition to white adipocytes, non-adipose tissues also reportedly produce leptin. Bado et al. (1998) and Sobhani et al. (2000) have demonstrated leptin production in the stomachs of rats and humans, respectively. Hansen et al. (2008) showed that porcine enterocytes and colonocytes express both leptin and leptin receptor. Wang et al. (1998) revealed that leptin production occurs in rat skeletal muscle and cultured L6 myocytes in response to nutrient load. Therefore, it would be irrational to exclude the possibility that increased plasma leptin in response to food intake is predominantly derived from extra-adipose tissues. Given that the increase of postprandial circulating leptin depends on GC-receptor signaling as suggested by the present study, it is of interest to test whether GCs stimulate leptin production in the stomach, small intestine, colon, and skeletal muscle. In the present study, however, ELISA analysis failed to detect leptin in the culture supernatant of Caco-2 cells, even when the cells were cultured in the presence of GC-synthesis promoter (data not shown).

In conclusion, the present findings suggest that extra-adrenal GCs contribute to the GC-receptor signaling-dependent increase of postprandial circulating leptin. Although the present in vitro studies suggest that enterocyte-derived GCs stimulate leptin production in adipocytes through GC-receptor

signaling, further studies will be required to determine whether enterocytes participate in the GCs-mediated increase of postprandial circulating leptin in vivo.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to disclose.

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