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Original Article

Role of Hormone-sensitive Lipase in Leptin-Promoted Fat Loss and Glucose Lowering

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***Aim*: Myriad biological effects of leptin may lead to broad therapeutic applications for various metabolic diseases, including diabetes and its complications; however, in contrast to its anorexic effect, the molecular mechanisms underlying adipopenic and glucose-lowering effects of leptin have not been fully understood. Here we aim to clarify the role of hormone-sensitive lipase (HSL) in leptin's action.**

***Methods*: Wild-type (WT) and HSL-deficient (HSLKO) mice were made hyperleptinemic by two commonly-used methods: adenovirus-mediated overexpression of leptin and continuous subcutaneous infusion of leptin by osmotic pumps. The amount of food intake, body weights, organ weights, and parameters of glucose and lipid metabolism were measured.**

***Results*: Hyperleptinemia equally suppressed the food intake in WT and HSLKO mice. On the other hand, leptin-mediated fat loss and glucose-lowering were significantly blunted in the absence of HSL when leptin was overexpressed by recombinant adenovirus carrying leptin. By osmotic pumps, the fat-losing and glucose-lowering effects of leptin were milder due to lower levels of hyperleptinemia; although the difference between WT and HSLKO mice did not reach statistical significance, HSLKO mice had a tendency to retain more fat than WT mice in the face of hyperleptinemia.**

***Conclusions*: We clarify for the first time the role of HSL in leptin's effect using a genetic model: leptin-promoted fat loss and glucose-lowering are at least in part mediated via HSL-mediated lipolysis. Further studies to define the pathophysiological role of adipocyte lipases in leptin action may lead to a new therapeutic approach to circumvent leptin resistance.**

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Key words: Leptin, Lipolysis, Lipase, Hypoglycemia, Leptin resistance

Introduction

Intraabdominal adiposity plays a key role in the pathogenesis of diabetes, dyslipidemia, and their complications such as arteriosclerosis. Leptin is an adipocyte hormone, which is secreted in response to lipid storage and regulates adipose tissue mass[1, 2]. The pivotal role of leptin in physiology is evident from the massive obese phenotype of mice and humans with recessive mutations in the leptin gene. Mainly acting in the brain[3], leptin regulates energy metabolism, by inhibiting food intake, stimulating lipolysis in adipocytes, and enhancing insulin sensitivity in the periph-

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eral tissues. These multiple effects of leptin, i.e. anorexic, adipopenic, and glucose/insulin-lowering effects, suggest its potential therapeutic utility in a broad range of pathologic status, including obesity, diabetes, and their complications[4, 5]. In fact, leptin effectively improves glucose metabolism and other metabolic derangements in mouse models of leptin deficiency[6-8], lipodystrophies[9-11], type 1 diabetes[12-16], type 2 diabetes[17], as well as humans with congenital leptin deficiency[18, 19] and lipodystrophic diabetes[20, 21].

In contrast to the well-characterized effect of leptin on feeding behavior, the mechanisms underlying the adipopenic effects of leptin[22] are not fully understood. Adipopenic effect of leptin is partly independent of its anorexic effect, as suggested by the observations that hyperleptinemic mice and rats lose body fat considerably more than their pair-fed control animals[23-25]. It has been suggested that leptin, in addition to its anorexic effect, activates sympathetic nerve systems (SNS) innervating white adipose tissue (WAT) to increase lipolysis in adipocytes[26]. Zeng *et al*. recently proved this to be the case[27], by showing that genetic ablation of sympathetic inputs blocks leptin-stimulated lipolysis. They also demonstrated that leptin stimulates phosphorylation of hormone-sensitive lipase (HSL), a canonical triglyceride (TG) hydrolase in adipocytes, via SNS-catecholamine pathway; however, direct evidence is lacking whether HSL is necessary for the adipopenic effect of leptin.

The glucose/insulin-lowering effect of leptin is also suggested to be independent of leptin's anorexic effect[24, 28]. Efforts to narrow down the site in the central nervous system (CNS) that specifically mediates leptin's glucose/insulin-lowering effect have revealed the dominant contribution of the hypothalamic arcuate nucleus (ARH)[29-32] and ventromedial hypothalamic nucleus (VMH)-SNS-catecholamine pathway[33, 34], or possibly other sites[35]. In contrast, the "efferent" effectors by which CNS regulates glucose metabolism are largely unknown[36]. Peroxisome proliferator-activated receptor (PPAR)α pathway is one of such candidates, as the adipopenic effect, as well as the glucose/insulin lowering effect of leptin, is abolished in PPARα-deficient (PPARα−/−) mice[37]. Another candidate would be the molecule(s) downstream of SNS-catecholamine pathway such as adipocyte lipases because SNS is supposed to mediate both adipopenic[27] and glucose-lowering effects[33, 34] of leptin; however, the contribution of adipocyte lipase(s) in leptin's glucose-lowering action has never been tested directly.

Aim

The purpose of this study is to define the role of HSL in the anorexic, adipopenic, and glucose-lowering effects of leptin using gene-targeted mice deficient in HSL (HSLKO). We followed the commonly-used methods of hyperleptinemia, i.e., injection of recombinant adenovirus carrying leptin (Ad-Leptin)[24] and continuous subcutaneous infusion of recombinant leptin by osmotic pumps[23], which produces supraphysiological and near-physiological levels of hyperleptinemia, respectively. We clarify, for the first time, the role of HSL in leptin's effects on fat loss and glucose lowering.

Methods

Animals

HSL-deficient (HSLKO) mice[38], which were backcrossed at least five times into the C57BL/6J background, were used in this study. Genotyping was performed as described previously[38]. Mice were housed in a temperature-controlled environment with a 12-h light/dark cycle and were allowed free access to water and a standard chow diet (Oriental MF, Oriental Yeast, Tokyo, Japan; CLEA Rodent Diet CE-2, CLEA Japan, Tokyo, Japan). Mice were maintained, cared for, and used in experiments in accordance with the regulations of the Animal Care Committees of the University of Tokyo.

Construction of Recombinant Adenoviruses

Recombinant adenovirus carrying mouse leptin cDNA under the control of the cytomegalovirus promoter, designated as Ad-Leptin, was constructed using the cDNA cloned by reverse transcription polymerase chain reaction (RT-PCR) from mouse liver as described previously[39]. Recombinant adenovirus containing the β-galactosidase cDNA (Ad-LacZ) was used as a control. The recombinant adenoviruses were expanded in HEK293 cells and purified by cesium chloride ultracentrifugation. The purified viruses were stored in 10% (v/v) glycerol in phosphate buffered saline (PBS) at −80℃. In our preparations, 1 multiplicity of infection (m.o.i.) corresponded to 25 particles of adenovirus per cell.

Adenovirus Experiments

Mice (8−12 weeks, 4 mice in each group) were injected intravenously with 1.0×10^{11} particles (4×10^{9} plaque-forming units) of Ad-Leptin, or, as a control, Ad-LacZ. Seven days after virus injection, food was withdrawn 5 h before collection of blood samples from the retro-orbital plexus of anesthetized animals. Tissues were immediately collected, snap frozen in liquid nitrogen, and stored at −80℃. The experiment was initially performed in female mice and then

repeated in male mice to confirm the results.

Subcutaneous Leptin Infusion

Miniosmotic pumps (ALZET #1003D, #1007D, DURECT, CA) were loaded with mouse recombinant leptin (rmLeptin, #121-05041, Wako (Lot#SAK0534, #SAQ1397)), which were designed to deliver leptin at a rate of 0.5 μg/1 μL/h (#1003D) or 0.5 μg/0.5 μL/h (#1007D) over a 3-day (#1003D) or 7-day (#1007D) period. Pumps were implanted subcutaneously between the scapulae of each mice (9−11 weeks old, 4 male mice in each group). The untreated control group received PBS delivered by the same osmotic pumps. Amounts of food intake, body weights, and blood glucose levels were monitored daily. On day 3 (#1003D) or day 7 (#1007D) after the implantation of miniosmotic pumps, mice were sacrificed and tissues were harvested.

Biochemical Analyses

Plasma levels of glucose were measured by ANTSENSE II (Bayer Medical, Tokyo, Japan) or by Glutest Neo (Sanwa Kagaku Kenkyusho; Mie, Japan). Plasma levels of leptin and insulin were assayed with the mouse leptin and insulin enzyme-linked immunosorbent assay (ELISA) kits (Morinaga, Tokyo, Japan). Plasma levels of cholesterol (Determiner TC; Kyowa Medex, Tokyo, Japan), trigycerides (TG) and glycerol (TG LH; Wako Chemicals, Tokyo, Japan), and free fatty acids (NEFA C; Wako Chemicals) were measured enzymatically.

Statistical Analyses

All values are given as mean ± standard error (SE). Differences between groups were evaluated with Student's *t*-test, one-way or two-way ANOVA by STAT view, version 5.0, for Macintosh (SAS Institute), or by PRISM 5 for Mac OS X (GraphPad Software, Inc.).

Results

HSL Does Not Contribute to the Anorexic Effect of Leptin

In order to define the role of HSL in leptin's action, we first took advantage of the model of adenovirus-mediated overexpression of leptin. We generated adenovirus carrying mouse leptin (Ad-Leptin) or β-galactosidase (Ad-LacZ), which were injected into wild-type (WT) or HSL-deficient mice (HSLKO). Compared with Ad-LacZ treated mice, Ad-Leptin treatment elicited substantial increases in the plasma leptin levels in both genotypes of mice with no difference between genotypes (WT-LacZ: 1.2 ± 0.6 ng/mL; WT-Leptin: 595 ± 114 ng/mL; HSLKO-LacZ: 0.67 ± 0.07 ng/mL; HSLKO-Leptin: 554 ± 54 ng/mL). Consequently, Ad-Leptin treatment suppressed food intake to a similar degree in both genotypes (**Fig. 1A**). The transient inhi-

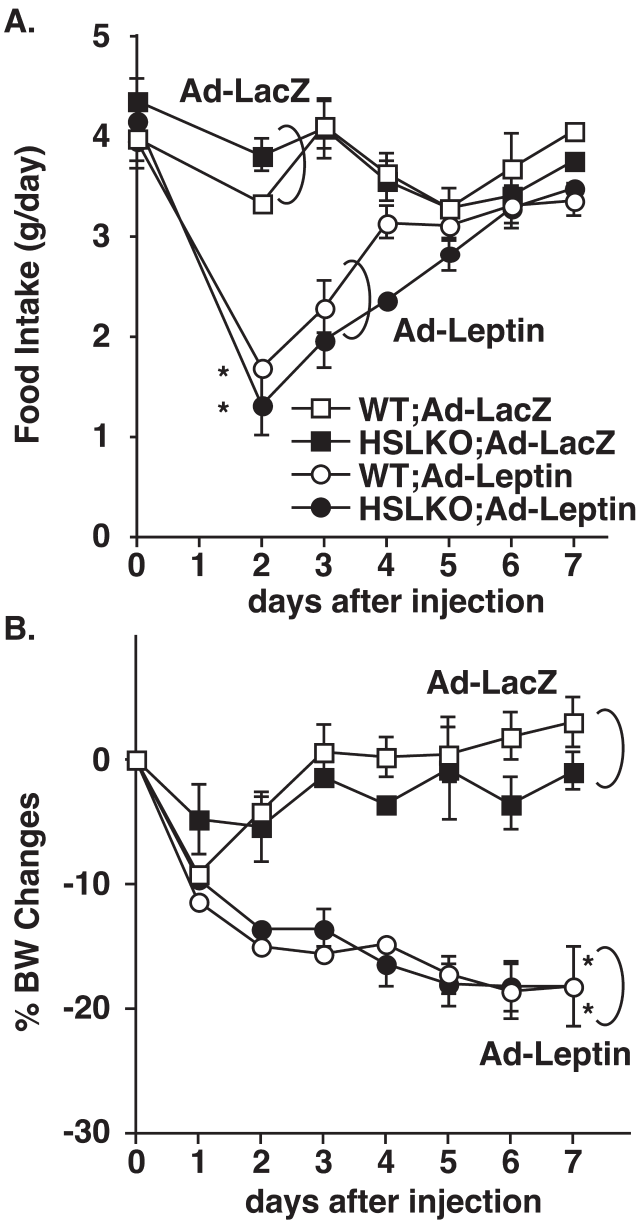

Fig. 1. Effect of Ad-Leptin on food intake and body weight in wild-type (WT) and HSLKO mice.

Amounts of food intake (A), and body weights (BW) were measured in WT (□, ○) and HSLKO (■, ●) mice that received infusions of Ad-Leptin, or Ad-LacZ (8−12 weeks old, 4 female mice in each group). Body weight is expressed as the percent difference from the body weight on day 0 of baseline measurement (B). Baseline body weights were not different among groups (WT-LacZ (□), 18.5 ± 1.0 g; WT-Leptin (○), 18.8 ± 1.0 g; HSLKO-LacZ (■), 20.4 ± 0.7 g; HSLKO-Leptin (●), 19.3 ± 0.1 g). Each value represents the mean ± SE of data from 4 mice. *, $P < 0.05$, Ad-LacZ versus Ad-Leptin, by two-way ANOVA.

bition of food intake after hyperleptinemia was consistent with the previous reports[24, 40]. Body weight, which was similar between WT and HSLKO mice at the start of the experiment (WT: 18.6 ± 0.7 g; HSLKO: 19.9 ± 0.4 g), declined progressively in both genotypes to a similar degree (**Fig. 1B**). These data verified the efficiency of the method to overexpress leptin by adenovirus and revealed no difference between WT and HSLKO in terms of the anorexic effect of leptin.

HSL Contributes to the Leptin's Effect on Fat Loss

Previous works have demonstrated that leptin has a specific adipopenic effect, which depletes adipose tissues completely in Ad-Leptin-induced hyperleptinemia[24, 28, 37, 41]. We next tested if this adipopenic effect of leptin requires HSL. After the injection of Ad-Leptin, there was a striking difference between genotypes in the appearance and weight of fat pads (**Fig. 2**). Consistent with previous reports[24], hyperleptinemia resulted in the disappearance of visible fat in WT mice (**Fig. 2A**), and the weight of parametrial white adipose tissue (WAT) declined from 104 to 6 mg (−94.5%) (**Fig. 2B**). In clear contrast, HSL-deficient mice retained substantial amounts of fat pads (**Fig. 2A**), and the weight of the parametrial fat declined from 154 to 26 mg (−83%) (**Fig. 2B**). Compared to WT mice treated with Ad-Leptin, HSLKO mice treated with Ad-Leptin retained 4.3 times more fat pads (**Fig. 2B**, $P<0.05$). Similarly, subcutaneous fat remained significantly more ($P<0.01$) in HSLKO;Ad-Leptin mice than in WT;Ad-Leptin mice (WT-LacZ: 186 ± 30 mg; WT-Leptin: 40 ± 3 mg; KO-LacZ: 233 ± 38 mg; HSLKO-Leptin: 59 ± 3 mg). In contrast to WAT, leptin treatment reduced the weight of brown adipose tissue (BAT) in both genotypes similarly with no difference between Ad-leptin treated WT and HSLKO (WT-leptin: 25 ± 2 mg; HSLKO-leptin: 28 ± 2 mg) (**Fig. 2C**). These results suggest that the adipopenic effect of leptin in WAT is mediated at least in part via the HSL-mediated lipolytic pathway.

HSL Contributes to the Leptin's Effect on Glucose Lowering

We next tested if the metabolic effect of leptin is HSL dependent as well. Previous works have suggested that leptin has its specific effect on glucose metabolism, such as enhancing insulin sensitivity, at least partially independent of its anorexic effect[24, 28-30, 36, 42]. As shown in **Fig. 3A**, plasma levels of glucose in *ad lib* gradually fell in WT mice after Ad-Leptin injection ($P<0.05$, WT-LacZ vs. WT-Leptin), but were only moderately and non-significantly reduced in HSLKO mice. Plasma glucose levels on day 7 *ad lib* (**Fig. 3A**) were significantly decreased only in WT mice (WT-LacZ: 176 ± 11 mg/dL; WT-Leptin: 87 ± 11 mg/dL; HSLKO-LacZ: 154 ± 6 mg/dL; HSLKO-Leptin: 134 ± 22 mg/dL). The difference in parameters of glucose metabolism between WT and HSLKO mice was more striking when they were fasted for 5 h on day 7 (**Fig. 3B**, **3C**). Upon fasting, glucose levels dropped severely from 87 mg/dL (before fasting (**Fig. 3A**)) to 11 mg/dL (fasting (**Fig. 3B**)) in WT mice treated with Ad-Leptin (**Fig. 3B**). Concomitantly, plasma insulin levels fell dramatically in Ad-Leptin treated WT mice, most likely due to a compensatory response to hypoglycemia (**Fig. 3C**). In clear contrast, plasma glucose levels reduced only moderately from 134 mg/dL (before fasting (**Fig. 3A**)) to 57 mg/dL (fasting (**Fig. 3B**)) in HSLKO mice treated with Ad-Leptin (**Fig. 3B**), without significantly changing plasma insulin levels (**Fig. 3C**). Consistent with previous reports including ours[38, 43, 44], fasting plasma free fatty acids (FFA) levels were reduced in HSLKO than WT mice of the control group injected with Ad-LacZ ($P<0.05$; **Fig. 3D**). Leptin treatment declined plasma FFA levels from 346 to 95 μM (−73%) in WT mice ($P<0.05$), but only moderately from 238 to 158 (−34%) in HSLKO mice. The plasma FFA levels most likely reflect the amounts of fat, a major source of plasma FFAs in the body (**Fig. 3D**). Other lipid parameters, such as TG (**Fig. 3E**) and total cholesterol (data not shown) were reduced by leptin treatment similarly in both genotypes without any difference between the genotypes. The fall in plasma FFAs and TG after Ad-Leptin treatment in WT mice was consistent with the previous reports[24, 37]. These results suggest that leptin's effect on glucose metabolism was at least partially dependent on HSL.

Contribution of HSL Depends on the Levels of Hyperleptinemia

These data so far clearly demonstrate that HSL contributes to the leptin's effect on fat loss and glucose lowering, but not on food intake (**Figs. 1**, **2**, and **3**), in the setting of the supra-physiological levels of hyperleptinemia that induce severe fat loss. We next tested if HSL plays a role in leptin actions at near-physiological concentrations of hyperleptinemia. To this end, we utilized an osmotic pump model to continuously infuse leptin subcutaneously[28]. As shown in **Fig. 4A**, infusion of leptin successfully increased plasma levels of leptin in both genotypes (WT-PBS: 0.7 ± 0.4 ng/mL; WT-Leptin: 20.9 ± 1.7 ng/mL; HSLKO-PBS: 1.6 ± 0.7 ng/mL; HSLKO-Leptin: 19.9 ± 1.0 ng/mL), with no significant difference between the genotypes. Food intake was suppressed similarly in WT and HSLKO mice ($P<0.05$), confirming again that HSL does not contribute to the anorexic effect of leptin

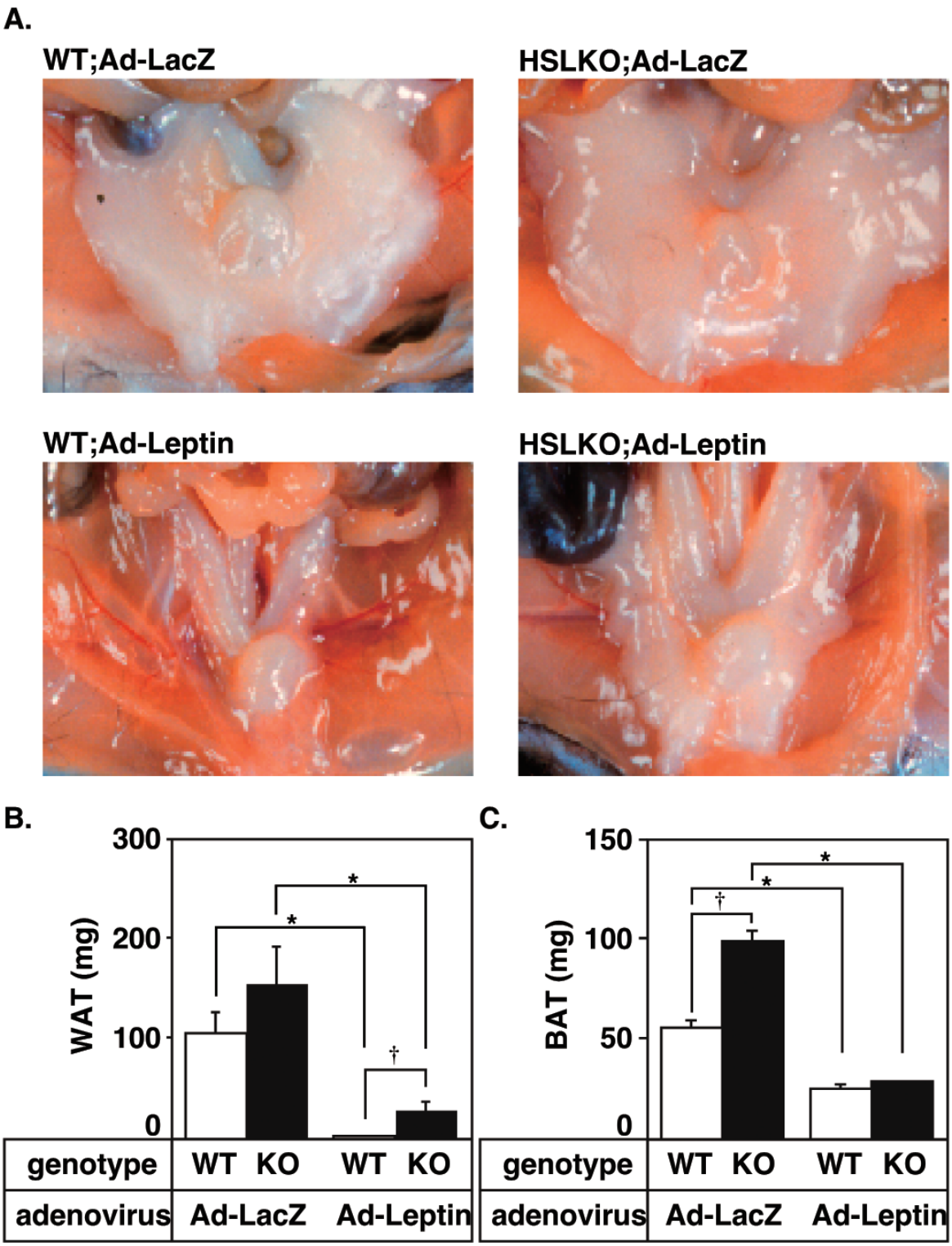

Fig. 2. Effects of Ad-Leptin on the appearance of adipose tissues and organ weights in wild-type (WT) and HSL-deficient (KO) mice.

Seven days after intravenous administration of Ad-Leptin, or Ad-LacZ as a control, in WT and HSLKO mice (8–12 weeks old, 4 female mice in each group), the appearance of parametrial white adipose tissue (WAT) (A) was compared and organ weights of parametrial WAT and brown adipose tissue (BAT) (B) were measured. Each value represents the mean ± SE of data from 4 mice. *, $P < 0.05$, Ad-LacZ versus Ad-Leptin, by one-way ANOVA; †, $P < 0.05$, WT versus HSLKO, by one-way ANOVA.

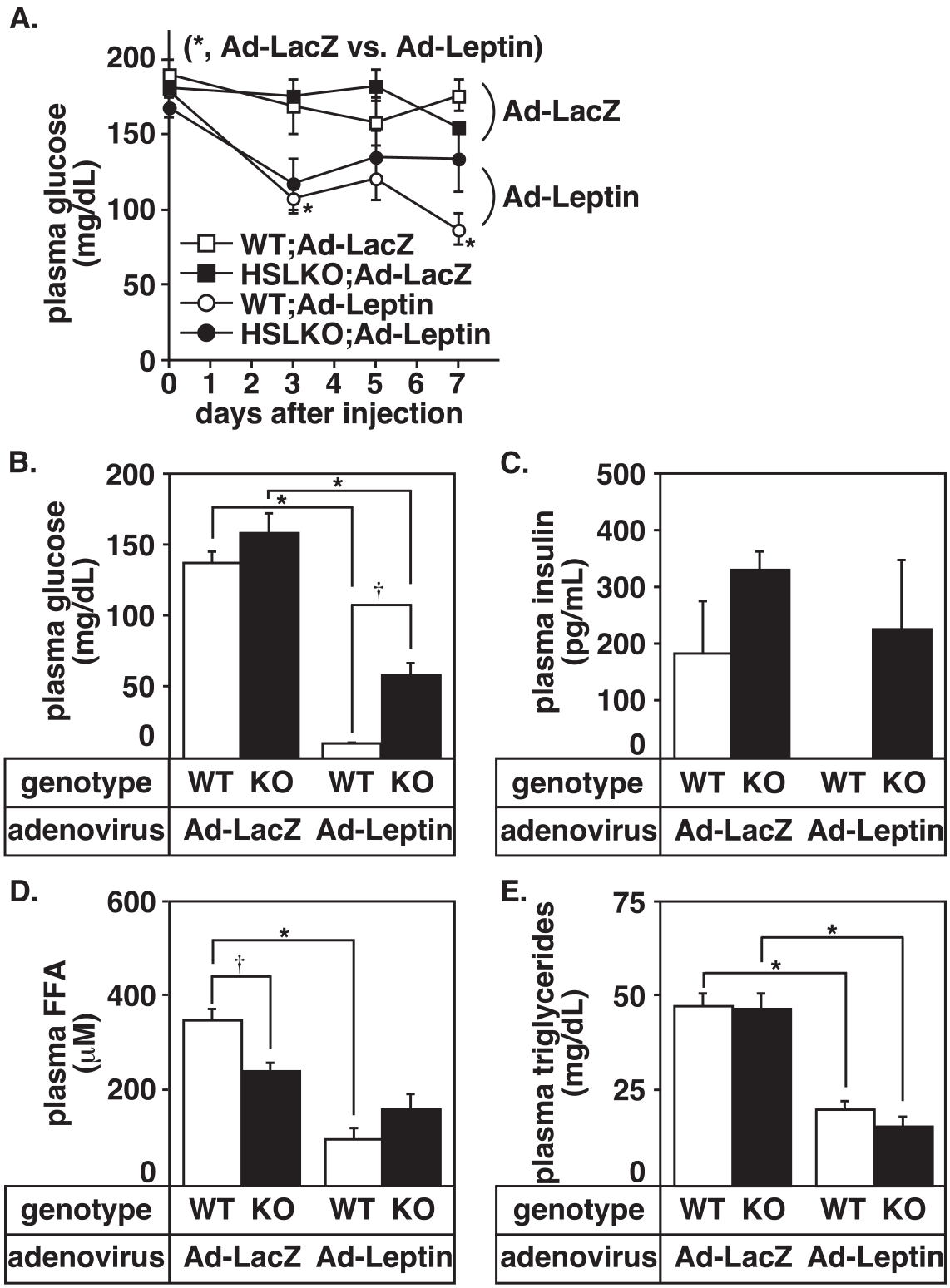

Fig. 3. Effects of Ad-Leptin on plasma metabolic parameters in wild-type (WT) and HSL-deficient (KO) mice.

After intravenous administration of Ad-Leptin or Ad-LacZ as a control in WT (□, ○) and HSLKO (■, ●) mice (8−12 weeks old, 4 female mice in each group), levels of plasma glucose *ad lib* at the indicated time points (A), and fasting levels of plasma glucose (B), insulin (C), free fatty acids (FFA) (D), and triglycerides (TG) (E) on day 7 were measured enzymatically. Each value represents the mean ± SE of data from 4 mice. *, $P < 0.05$, Ad-LacZ versus Ad-Leptin, by one-way ANOVA; †, $P < 0.05$, WT versus HSLKO, by one-way ANOVA.

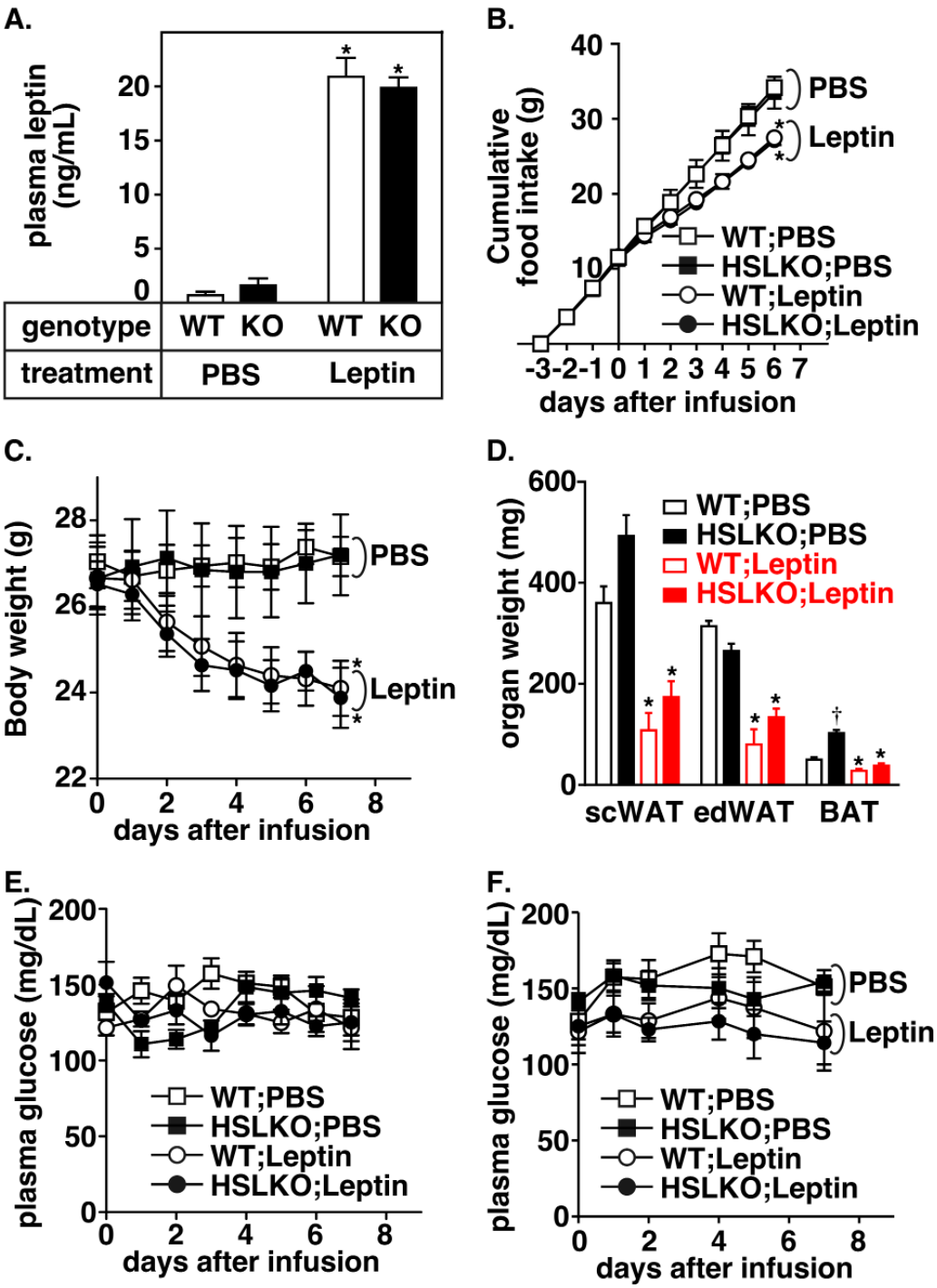

Fig. 4. Effects of leptin infusion by osmotic pumps on metabolic profiles in wild-type (WT) and HSL-deficient (KO) mice.

Osmotic minipumps (ALZET # 1007D, DURECT, CA) containing either leptin (1 mg/mL) or vehicle (PBS) were implanted subcutaneously in WT (□, ○) and HSLKO (■, ●) mice (9−11 weeks old, 4 male mice in each group). Plasma leptin levels at 7 days after the implantation were determined by ELISA (A). Amounts of food intake (B) and body weights (C) were measured at the indicated time points, and organ weights of epididymal white adipose tissue (edWAT), subcutaneous WAT (scWAT), and brown adipose tissue (BAT) were measured on day 7 (D) after the implantation. Plasma glucose levels were tested *ad lib* at the indicated time points (E) or in the fasted status on day 7 after the pump implantation (F). Each value represents the mean ± SE of data from 4 mice. *, $P<0.05$, Ad-LacZ versus Ad-Leptin, by one-way ANOVA (A and F), or by two-way ANOVA (B and C); †, $P<0.05$, WT versus HSLKO, by one-way ANOVA.

(**Fig. 4B**). In parallel with the suppressed food intake, body weights were reduced significantly after leptin infusion both in WT and HSLKO mice ($P < 0.05$, **Fig. 4C**). At this levels of hyperleptinemia, body fat reduced significantly in WT mice (WT-PBS, 314 ± 1 mg; WT-leptin, 80 ± 3 mg) (**Fig. 4D**), but not as completely as in the Ad-Leptin model (**Fig. 2B**). Although HSLKO tended to retain more fat than WT mice after leptin infusion, the difference between genotypes did not reach statistical significance both in epididymal WAT (edWAT) (WT-Leptin: 80 ± 3 mg; HSLKO-Leptin: 134 ± 17 mg) and subcutaneous WAT (scWAT) (WT-Leptin: 108 ± 35 mg; HSLKO-Leptin: 174 ± 31 mg)(**Fig. 4D**). At this level of hyperleptinemia, leptin treatment did not significantly reduce the plasma levels of glucose in both genotypes, either *ad lib* (**Fig. 4E**) or in fasted status (**Fig. 4F**). These results suggest that HSL-mediated lipolytic pathway contributes to leptin's effect on fat loss and glucose-lowering more dominantly at the higher levels of hyperleptinemia that cause almost complete fat loss (**Figs. 1**, **2**, and **3**) than at the lower levels of hyperleptinemia in otherwise healthy mice (**Fig. 4**).

Discussion

Leptin contributes to the homeostasis of body fat by acting on a myriad of metabolic pathways, and leptin therapy is increasingly being used in a variety of disorders in humans. A precise understanding of the biological actions of leptin is warranted. Mainly acting in the brain, leptin inhibits food intake, stimulates adipocyte lipolysis[23, 24, 27], and improves glucose metabolism[24, 28-30]. The latter two effects are at least in part independent of its anorexic effect; however, the downstream effectors that increase adipocyte lipolysis and improve glucose metabolism have not been fully understood[27, 36, 37]. The report herein identified HSL, an adipocyte TG lipase, as an efferent effector that partly confers the effect of leptin on fat loss (**Fig. 2**) as well as glucose lowering (**Fig. 3**), but not on suppression of food intake (**Fig. 1**). We also found that the role of HSL is more dominant at supra-physiological hyperleptinemia that elicited complete fat loss in WT mice (**Fig. 2**) than at near-physiological hyperleptinemia where the adipose depletion in WT is incomplete (**Fig. 4**). To our knowledge, this is the first study to clarify the role of adipocyte lipase in leptin's effect using a gene-targeted mouse model.

Compared to the well-defined action of leptin in regulating food intake, the molecular mechanisms underlying leptin's adipopenic effect are not fully understood. Previous work by Chen *et al.* found that leptin has a "specific" adipopenic effect, which is at least partially independent of its anorexic effect[24]. By comparing Ad-Leptin treated rats versus Ad-LacZ treated rats pair-fed to Ad-Leptin treated rats, they found that Ad-Leptin treated rats lost body fat almost completely, whereas the pair-fed rats retained about 50% of the body fat[24]. We used the same adenovirus model (**Fig. 1**), and demonstrated for the first time that this "specific" adipopenic effect of leptin (**Fig. 2**) is at least in part mediated by HSL. Adipopenic effect of HSL is independent of leptin's anorexic effect, as leptin similarly suppressed food intake both in WT and HSLKO mice (**Fig. 1**). Unger's group previously reported a similar phenomenon in PPARα deficient mice: They found that hyperleptinemia by Ad-Leptin depleted the fat mass in WT mice but not in PPAR$\alpha^{-/-}$ mice, despite the same degree of suppression of food intake in WT and PPAR$\alpha^{-/-}$ mice[37]. As leptin upregulates mRNAs of PPARα and its target genes of fatty acid (FA) oxidation, they conclude that PPARα-mediated oxidation of FA is necessary for the adipopenic action of leptin. It is likely that leptin, on the one hand, stimulates lipolysis by activating adipocyte lipase(s) to liberate FAs, and on the other hand activates PPARα pathway to oxidize FAs, collectively leading to the adipose depletion.

The mechanism that leptin stimulates lipolysis has only recently been uncovered at the molecular level. Zeng *et al.* recently reported that leptin increases the phosphorylation of HSL and stimulates lipolysis in adipocytes, via sympathetic nerve fibers that innervate the adipose tissue[27]. Disruption of sympathetic inputs to the fat pads either genetically, surgically, or pharmacologically, almost completely blocked leptin-stimulated phosphorylation of HSL. The role of SNS-catecholamine pathway in the leptin-mediated fat loss was further proved *in vivo* using mice deficient in dopamine β-hydroxylase (DBH$^{-/-}$): delivery of leptin by osmotic pumps at a rate of 0.5 μg/h reduced fat in the wild-type mice but not in the DBH$^{-/-}$ mice[27]. To compare the role of HSL with that of DBH in this context, we used exactly the same experimental condition in our experiments in **Fig. 4**. Although adenovirus model clearly demonstrated the contribution of HSL in leptin-mediated fat loss (**Fig. 2**), the osmotic pump model (0.5 μg/h for 7 days, Alzet #1007D) revealed only a nonsignificant tendency that HSLKO mice retain more fat than WT mice (**Fig. 4D**). We confirmed this result by repeating experiments using a different model of osmotic pumps for a shorter duration (0.5 μg/h for 3 days, Alzet #1003D). The difference between the two models could be due to the different route of leptin release: from the liver for the adenovirus model versus subcutaneous tissues for the osmotic pump model. Alternatively, the difference may

result from the different levels of hyperleptinemia: ~550−600 ng/mL for the adenovirus model (as described in Results) versus ~20 ng/mL for the osmotic pump model (**Fig. 4A**). A likely explanation would be that HSL plays a significant role at higher levels of leptin, and other adipocyte lipases may play a more dominant role at lower levels of leptin. As adipocyte lipolysis is mediated not only by HSL but also by ATGL[45], TGH-1[46], or TGH-2[47], these lipases may play a dominant role in leptin-mediated lipolysis and fat loss downstream of SNS-DBH-catecholamine pathway. In this sense, it is of note that ATGL contributes more dominantly than HSL to cancer-associated cachexia, another model of severe fat loss[48]. Interestingly, our data demonstrate that the contribution of HSL seems more dominant in WAT than in BAT (**Figs. 2C** and **4D**), suggesting that the contribution of HSL and ATGL may differ in different types of adipose tissues. The fact that ATGL knockout, but not HSL knockout, is cold sensitive[38, 49], and the fact that TG lipase activity is decreased in WAT (by 60%) but not in BAT of HSLKO mice[38], may suggest that ATGL plays a more dominant role in BAT. Further studies are warranted to clarify the contribution of each adipocyte lipase in the leptin-mediated fat loss.

Then, how leptin coordinately increases lipolysis via SNS and at the same time increases FA oxidation via PPARα? Increasing evidence suggests the physiological importance of lipolysis-PPAR axis: lipolysis activates PPARs by providing cognate ligand for PPARs. For example, ATGL-PPAR axis controls myriads of metabolic pathway in a variety of tissues: FAs derived from ATGL-mediated lipolysis regulate mitochondrial function in the heart via PPARα/PGC-1[50], maintain mitochondrial function in muscle via PPARα[51, 52], promote mitochondrial function for insulin secretion in islet β cells via PPARδ[53], activate PPARα in hepatocytes[54], regulate intestinal lipid metabolism via PPARα[55], and regulate FA oxidation in BAT via PPARα and PPARδ[56]. HSL-PPAR axis controls lipogenesis and adipogenesis in adipocytes via PPARγ[57-60]. The importance of HSL-PPAR axis in human physiology is recently highlighted from the discovery of human HSL null patients, who have partial lipodystrophic and diabetic phenotype, accompanying the downregulation of PPARγ and its downstream target genes in adipose tissues[61].

The role of HSL in leptin's action is also suggested from the contribution of HSL in leptin-mediated glucose lowering (**Fig. 3**). In WT mice, Ad-Leptin improved glucose metabolism (**Fig. 3A**), which largely confirms the previous results[24, 37]. We also found that the glucose-lowering effect of Ad-Leptin was more striking when mice were fasted (**Fig. 3B**), suggesting that Ad-Leptin induced hypoglycemia by blocking gluconeogenesis. Currently, the precise mechanisms underlying leptin-induced fasting hypoglycemia in the adenovirus model is unclear. Changes in the counter-insulin hormones or transcription of gluconeogenic genes could not explain the fasting hypoglycemia of hyperleptinemic mice; we found rather increased levels of counter-insulin hormones such as glucagon and corticosterone, and increased mRNA levels of gluconeogenic genes, such as PGC1α, G6Pase, and PEPCK, in WT mice treated with Ad-Leptin, most likely as compensatory responses to hypoglycemia (data not shown). Decreased availability of substrates for gluconeogenesis is another possibility; however, our preliminary data indicate that leptin-induced hypoglycemia is not rescued by supplying substrates for gluconeogenesis (unpublished observations). Considering the protection against the leptin-induced hypoglycemia in HSLKO mice (**Fig. 3**), it can be hypothesized that some fat-derived factor(s) or lipolysis-derived factor(s), which may correlate with fat mass, affect gluconeogenesis in liver posttranslationally. The milder hypoglycemic effect in the face of milder fat-loss at lower levels of hyperleptinemia (**Fig. 4**) may support this hypothesis. We are currently working to test this hypothesis of fat-gluconeogenesis axis of leptin's action. Nonetheless, our data reveal for the first time that adipocyte lipase(s) mediate leptin's glucose-lowering effect at least partially. Despite the broad therapeutic possibilities of leptin to normalize hyperglycemia as well as to reduce hypoglycemia in type 1 diabetes as an adjunct to insulin[15], leptin may have a potential adverse effect of severe hypoglycemia[16]. Further studies are needed to precisely define the molecular mechanisms of leptin-mediated glucose lowering.

The major limitation of the current study is that we could not rule out the possibility that the observed phenotype in HSLKO mice is not due to the loss of HSL *per se*, but due to some changes secondary to HSL deficiency. For example, the protection from leptin-induced hypoglycemia in HSLKO mice could be secondary to the changes in fat mass as discussed in the aforementioned paragraph, although the change in fat mass comes from the presence or absence of HSL. The observed phenotype in HSLKO mice could also be secondary to some changes in gene expression coupled to HSL deficiency. For example, mRNA expression of ATGL is about 70% lower in WAT of HSLKO mice than WT mice[62], which is reproducible in our HSLKO mice as well (~88% lower than WT mice, Takanashi M., unpublished results). The lower expression of ATGL could potentially contribute to the phenotype in HSLKO mice. The exact contribution of each adipocytes lipase will be addressed in future stud-

ies using inducible, tissue-specific knockouts of these lipases, which is beyond the scope of this study. Nonetheless, our study is the first to clarify the role of HSL in the leptin-mediated fat loss and glucose lowering, opening up a fruitful area of research.

The study herein aimed to clarify the role of HSL in leptin's action at therapeutic doses. Our data demonstrate that HSL contributes to the adipopenic and glucose-lowering effect of leptin more dominantly at higher doses (**Figs. 2** and **3**). Next issue would be whether HSL confers the sensitivity to leptin in normal physiology or some pathological conditions, such as lipodystrophy, or type 1 and type 2 diabetes. Although we could not detect a significant contribution of HSL at a near-physiological dose of leptin in otherwise healthy mice (**Fig. 4**), this issue should be tested in other pathological models of obesity or diabetes. Decreased lipolytic activity may lead to obesity in the face of hyperleptinemia, so called leptin resistance, or may compromise the effect of leptin therapy[63]. Conversely, stimulation of lipolysis (e.g., by direct activation of sympathetic inputs to adipose tissues[27]) may offer an alternative approach to induce fat loss and circumvent leptin resistance, a common feature of obesity.

Conclusion

Our data, for the first time, demonstrate that HSL contributes to leptin-mediated fat loss and glucose lowering. Future studies are warranted to elucidate the contribution of HSL or other adipocyte lipases such as ATGL in the physiological and therapeutic actions of leptin, for better understanding and treatment of diseases, such as lipodystrophy, diabetes, and its complications.

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Conflict of Interest

None.

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