



Hormone-sensitive lipase deficiency suppresses insulin secretion from pancreatic islets of *Lep^{ob/ob}* mice

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ABSTRACT

It has long been a matter of debate whether the hormone-sensitive lipase (HSL)-mediated lipolysis in pancreatic β-cells can affect insulin secretion through the alteration of lipotoxicity. We generated mice lacking both leptin and HSL (*Lep^{ob/ob}/HSL^{-/-}*) and explored the role of HSL in pancreatic β-cells in the setting of obesity. *Lep^{ob/ob}/HSL^{-/-}* developed elevated blood glucose levels and reduced plasma insulin levels compared with *Lep^{ob/ob}/HSL^{+/+}* in a fed state, while the deficiency of HSL did not affect glucose homeostasis in *Lep^{+/+}* background. The deficiency of HSL exacerbated the accumulation of triglycerides in *Lep^{ob/ob}* islets, leading to reduced glucose-stimulated insulin secretion. The deficiency of HSL also diminished the islet mass in *Lep^{ob/ob}* mice due to decreased cell proliferation. In conclusion, HSL affects insulin secretory capacity especially in the setting of obesity.

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Introduction

Obesity and type 2 diabetes represent a growing threat to the health of the population of almost every country in the world. Accumulation of lipids in non-adipose tissues can lead to cell dysfunction, a phenomenon known as lipotoxicity as a molecular link between obesity and glucose homeostasis dysregulation [1]. Pancreatic β-cells are known to be highly susceptible to lipotoxicity, and both exogenous and endogenous sources of free fatty acids (FFAs) are believed to be involved in insulin secretory machinery [2,3]. The liberation of FFA by intracellular hydrolysis of acylglycerols, which is referred to as lipolysis, is observed in the pancreatic islets [4,5], and the lipolytic reaction supplies the endogenous FFA.

Hormone-sensitive lipase (HSL) is an intracellular neutral lipase that is capable of hydrolyzing triglycerides (TGs), diglycerides, monoglycerides, and cholesterol esters as well as other lipids

[6–9]. Although HSL was initially identified as an adipose-specific lipase, it has been clarified that HSL is also expressed and functions in a wide variety of organs and cells, including heart, skeletal muscle, adrenal glands, testes, ovaries, intestines and liver [7,8,10,11]. HSL was also identified in pancreatic β-cells [12], and several laboratories reported the relationship between the HSL-mediated lipolysis and insulin secretory machinery using rodent models without coming to consensus [13–17].

We reported the phenotypes caused by the combined deficiency of leptin and HSL (*Lep^{ob/ob}/HSL^{-/-}*) to explore the role of HSL in the setting of obesity [18]. Here we further explored the potential role of HSL in the insulin secretion using the leptin-deficient model. *Lep^{ob/ob}/HSL^{-/-}* showed elevated plasma glucose levels and decreased plasma insulin levels compared with *Lep^{ob/ob}/HSL^{+/+}* in a fed state, while the deficiency of HSL did not affect both plasma glucose levels and plasma insulin levels in a fasted state. In the *Lep^{+/+}* background, the glucose homeostasis was not affected by HSL. The impaired insulin secretion of *Lep^{ob/ob}/HSL^{-/-}* was further supported by glucose-stimulated insulin secretion (GSIS) from isolated islets. In addition, the deficiency of HSL decreased the enlarged islet mass in *Lep^{ob/ob}* mice due to decreased cell proliferation.

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Materials and methods

Animals. *Lep^{+/+}/HSL^{-/-}* and *Lep^{ob/ob}/HSL^{-/-}* mice were generated, and genotyping was performed as described previously [18,19]. Mice were housed in a temperature-controlled environment with a 12-h light/dark cycle and allowed free access to water and a standard chow diet (Oriental MF, Oriental Yeast, Tokyo, Japan). Mice were euthanized at 16 weeks after a 6-h fast. Blood was collected from the retro-orbital venous plexus after a 6-h fast (fasted state, at 8 and 16 weeks of age) or at the beginning of the light cycle (fed state, at 9 and 15 weeks of age). Plasma glucose was measured by ANTSENSE II (Bayer Medical, Tokyo, Japan), and plasma insulin was measured by the mouse insulin ELISA kit (Morinaga, Tokyo, Japan).

Pancreatic islets isolation and insulin secretion experiment. Islet isolation was carried out using the collagenase technique from 16-week-old non-fasted mice. In brief, 2.5 ml of collagenase (Type XI, Sigma, St. Louis, MO) solution at 4 mg/ml were introduced into the common bile duct after occlusion of the distal end just proximal to the duodenum. The distended pancreas was excised and the digestion was performed in a water bath at 37 °C for 3.5 min. The islets were washed and purified with a hand pick-up. Glucose stimulation tests were performed on isolated pancreatic islets essentially according to Sutton et al. [20]. Six groups (each group comprising 8 islets) were prepared from each mouse and three groups were used for low glucose (2.8 mM) stimulation and the other three were for high glucose (20 mM) stimulation. After pre-incubation at 37 °C for 30 min in KRBH buffer (130 mM NaCl, 5.2 mM KCl, 1.3 mM KH₂PO₄, 2.7 mM CaCl₂, 1.3 mM MgSO₄, 24.8 mM NaHCO₃, and 10 mM Hepes at pH 7.4) containing 2.8 mM glucose and 0.5% bovine serum albumin (BSA; Fraction V; Sigma, St. Louis, MO), groups of islets were incubated at 37 °C in KRBH containing 0.5% bovine serum albumin and either 2.8 mM glucose or 20 mM glucose for 30 min, then the media were withdrawn for insulin measurement. Lipids were extracted from ~100 islets/each mouse by chloroform-methanol method and TG content was measured by a kit (GPO-Trinder, Sigma, St. Louis, MO) (*n* = 8–11). Lipid extracted islets were dissolved in phosphate buffered saline by sonication. DNA content was measured by fluorescent method with Hoechst 33258 [21]. The size of islets under these experiments was controlled visually to be similar between *HSL^{+/+}* and *HSL^{-/-}* group.

Histology and quantification of islet mass. Sixteen-week-old mice were euthanized, and the pancreases were excised, fixed in 10% neutral buffered formalin, embedded in paraffin, and stained with hematoxylin-eosin. Three non-sequential sections from each pancreas were scanned (three mice for each genotype) by NIH image. At least 50 islets from each pancreas were quantified and total number of islets scanned was from 195 to 260 for each genotype.

Proliferating cells were detected by bromodeoxyuridine (BrdU) (Sigma, St. Louis, MO) immunostaining. Two hours after administration of BrdU intraperitoneally at 50 mg/kg body weight in phosphate buffered saline, mice were euthanized, and the pancreases were excised and embedded in paraffin. Immunostaining was performed by a BrdU *in situ* detection kit (PharMingen, San Diego, CA) and counterstained with Mayer's hematoxylin (Wako Pure Chemicals). Apoptotic cells were detected by terminal deoxynucleotidyl-transferase-mediated dUTP nick end-labeling (TUNEL) using an *in situ* apoptosis detection kit (Takara Biomedicals, Otsu, Japan) according to the manufacturer's instructions, with counterstaining with methyl green (Wako Pure Chemicals).

Statistics. Statistical differences between groups were analyzed by the Student's *t*-test or the one-way analysis of variance and a *post hoc* Tukey-Kramer test, unless otherwise stated.

Results and discussion

Levels of plasma insulin and glucose suggested the impaired insulin secretion in *Lep^{ob/ob}/HSL^{-/-}* mice

To gain insight into the physiological roles of HSL especially in the setting of obesity, we generated *Lep^{ob/ob}/HSL^{-/-}* mice. In this study, we attempted to characterize the roles of HSL in the pancreatic β-cells in the setting of obesity. In these mice, fasting plasma glucose and insulin levels were not affected by the HSL deficiency in both *Lep^{+/+}* and *Lep^{ob/ob}* backgrounds (Fig. 1). The results suggested the limited contribution of HSL to the whole-body insulin sensitivity, and in support of this finding, intraperitoneal insulin tolerance test was not affected by the HSL deficiency (data not shown). On the other hand, *Lep^{ob/ob}/HSL^{-/-}* mice showed elevated plasma glucose levels (26% increase for males and 72% increase for females) and decreased plasma insulin levels (45% decrease for males and 53% decrease for females) compared with *Lep^{ob/ob}/HSL^{+/+}* mice under fed conditions (Fig. 1), which suggested impaired insulin secretion in *Lep^{ob/ob}/HSL^{-/-}* mice. In the *Lep^{+/+}* background, there was no significant difference between *Lep^{+/+}/HSL^{+/+}* and *Lep^{+/+}/HSL^{-/-}* (Fig. 1). Since it was reported that the impaired insulin secretion of *HSL^{-/-}* mice was age- and gender-dependent [15], we examined both gender at two time points (8 weeks and 16 weeks for fasted conditions, and 9 weeks and 15 weeks for fed conditions. Data shown are representative of the two time points. It is also known that almost all the gender differences disappear in *Lep^{ob/ob}* mice). The *Lep^{ob/ob}/HSL^{-/-}* had defective insulin secretion even at an earlier age. We previously reported that the food intake was reduced in *Lep^{ob/ob}/HSL^{-/-}* mice [18], and the impaired insulin secretion of *Lep^{ob/ob}/HSL^{-/-}* might be underestimated under free-feeding conditions. In accordance with the impaired insulin secretion, the hepatic expression of gluconeogenetic enzymes such as phospho-enol-pyruvate carboxykinase (PEPCK) was reduced in *Lep^{ob/ob}/HSL^{-/-}* (data not shown).

Islets isolated from *Lep^{ob/ob}/HSL^{-/-}* mice displayed a decreased GSIS response

To eliminate the indirect effect, we isolated islets from each genotype, and GSIS assay was performed. In *Lep^{ob/ob}* background, the response of *Lep^{ob/ob}/HSL^{-/-}* islets to high concentration of glucose was significantly reduced by 36% compared with that of *Lep^{ob/ob}/HSL^{+/+}* islets (Fig. 2). In *Lep^{+/+}* background, the deficiency of HSL caused a milder decrease in GSIS, which did not reach statistical significance. The discrepancy between the earlier report [13] and our results needs to be studied further.

The deficiency of HSL led to marked accumulation of TG in *Lep^{ob/ob}* islets

Leptin deficiency may confer vulnerability to the lipotoxicity, because leptin causes TG depletion in islets via stimulating β-oxidation [22]. Furthermore leptin-treatment of *Lep^{ob/ob}* mice is known to reverse their glucose intolerance and is reported to upregulate HSL expression in islets [23], which imply protective function of HSL against lipotoxicity. Therefore, we examined whether and to what extent the deficiency of HSL influenced the islet TG content. There was a striking increase (78%) in islet TG content in *Lep^{ob/ob}/HSL^{-/-}* (Fig. 3A and C). The size of islets used for the GSIS experiment and lipid extraction was controlled visually to be similar between *HSL^{+/+}* and *HSL^{-/-}* group. To assure the precision, islet DNA content was measured fluorometrically (Fig. 3B), and the marked accumulation of islet TG in *Lep^{ob/ob}/HSL^{-/-}* was also supported by the data normalized with respect to DNA content

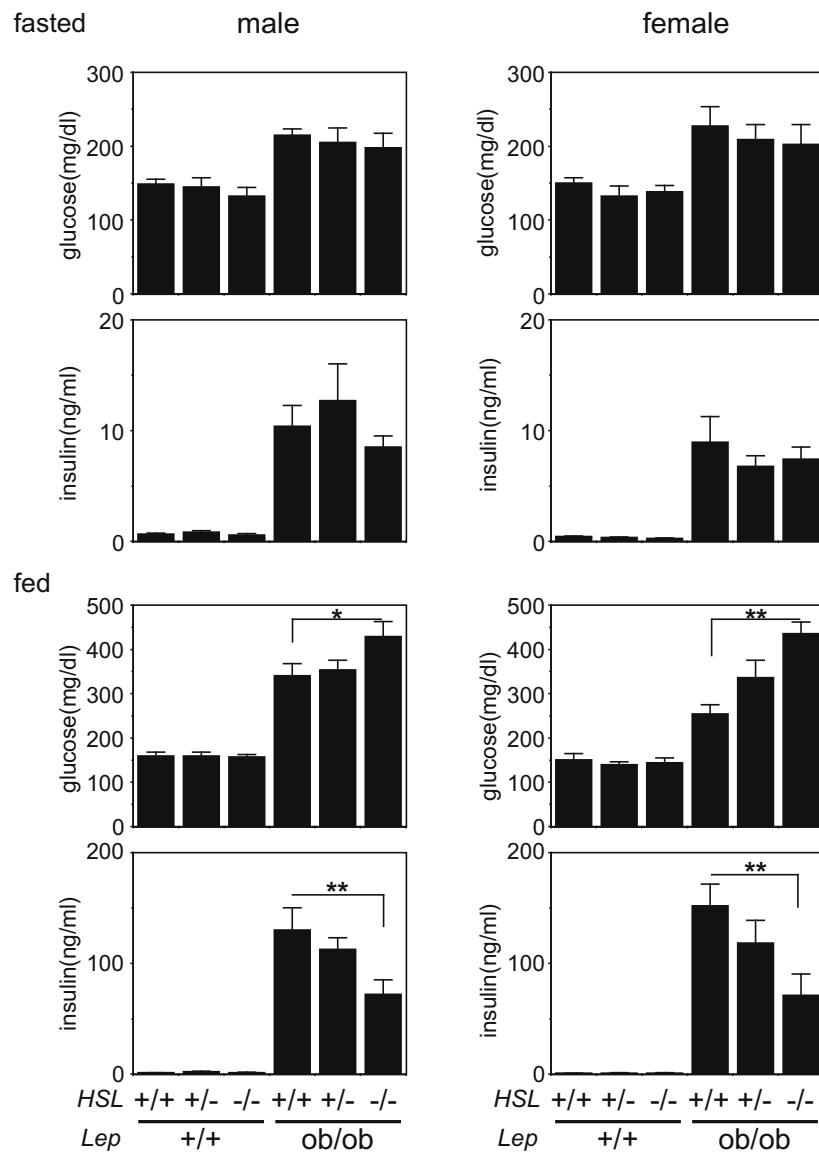


Fig. 1. *Lep*^{ob/ob}/*HSL*^{-/-} mice exhibited impaired insulin secretion *in vivo*. Blood samples were collected from either fasted (after a 6-h fast, at the age of 8 weeks, displayed in the upper four panels) or fed (at the beginning of light cycle, at the age of 15 weeks, displayed in the lower four panels) mice ($n = 10$). Each value represents the mean \pm SE. *And **denote significance at $P < 0.05$ and $P < 0.01$, respectively, vs *HSL*^{+/+} mice in the same *Lep* background determined by ANOVA followed by the Tukey–Kramer post hoc test. Similar pattern was observed in both younger (at the age of 8–9 weeks) and adult (at the age of 15–16 weeks) mice, and data shown are representative of the two time points.

(Fig. 3C). On the other hand, there was no significant difference between *Lep*^{+/+}/*HSL*^{+/+} and *Lep*^{+/+}/*HSL*^{-/-}, which is inconsistent with previous report [13]. Although it is necessary to further elucidate whether or not there might be a difference that was undetectable by our methods between *Lep*^{+/+}/*HSL*^{+/+} and *Lep*^{+/+}/*HSL*^{-/-}, the contribution of HSL to islet TG store was certainly clarified by the co-ablation of the leptin gene. Since excessive TG accumulation in islets is reported to require hyperglycemic background [24], HSL deficiency might induce lipotoxic impairment of insulin secretion only in the *Lep*^{ob/ob} background.

Islet mass was significantly reduced in Lep^{ob/ob}/*HSL*^{-/-}

Examination of pancreatic sections revealed that the islet mass was reduced by 25% in *Lep*^{ob/ob}/*HSL*^{-/-} compared with *Lep*^{ob/ob}/*HSL*^{+/+}, whereas there was no significant difference between *Lep*^{+/+}/*HSL*^{+/+} and *Lep*^{+/+}/*HSL*^{-/-} (Fig. 4A). Previous reports which addressed the reduced insulin secretory capacity in HSL deficient

mice showed no specific data regarding islet mass [13,15], while Holm and her colleagues reported the increased islet mass in HSL deficient mice accompanied by the whole-body insulin resistance [16]. Taken together with the findings in previous reports, the role of HSL in islet function would be limited, and the experiments using HSL deficient mice could sometimes produce confusing results. Our investigation using co-ablation of leptin gene would facilitate our understanding of the role of HSL in pancreatic β -cells. The combined effects of the reduction of insulin secretion per same size islet and reduced islet mass in *Lep*^{ob/ob}/*HSL*^{-/-} would impair insulin secretion *in vivo*.

Reduction in islet mass in Lep^{ob/ob}/*HSL*^{-/-} occurred via defective cell proliferation

To determine whether the decreased islet mass is due to defective proliferation or increased apoptosis, pancreatic sections were analyzed by immunohistochemistry for apoptosis by TUNEL

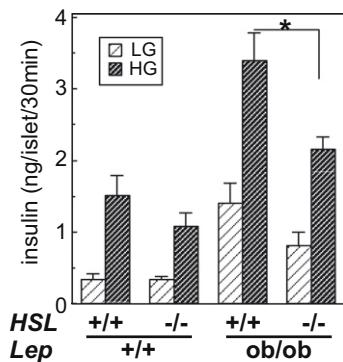


Fig. 2. Glucose-stimulated insulin secretion (GSIS) from *Lep*^{ob/ob}/*HSL*^{-/-} islets was impaired. Six groups (each group comprising 8 islets) were prepared from each mouse and three groups were used for low glucose (LG, 2.8 mM) stimulation and the other three were used for high glucose (HG, 20 mM) stimulation ($n = 5$ for each genotype). After preincubation at 37 °C for 30 min in KRBH containing 2.8 mM glucose, groups of islets were incubated at 37 °C in KRBH containing either 2.8 mM glucose or 20 mM glucose for 30 min, then the media were withdrawn for insulin measurement. Each value represents the mean \pm SE. *Denotes significance at $P < 0.05$, vs *HSL*^{+/+} mice in the same *Lep* background determined by the Student's *t*-test.

(Fig. 4B) and for cell proliferation by BrdU incorporation (Fig. 4C). No TUNEL positive nuclei were detected in pancreatic sections of all genotypes. On the other hand, BrdU incorporation study revealed that increased proliferative activity of *Lep*^{ob/ob}/*HSL*^{+/+} islets completely disappeared in *Lep*^{ob/ob}/*HSL*^{-/-} islets. The mechanism of upregulated proliferation of islet cells of *Lep*^{ob/ob} mice is not established [25], but this is believed to be due to the adaptation to the increased insulin demand [26]. The promoted

islet cell proliferation in *Lep*^{ob/ob}/*HSL*^{+/+} was completely abolished and no BrdU positive cells were detected in the sections of *Lep*^{ob/ob}/*HSL*^{-/-} as well as *Lep*^{+/+}/*HSL*^{+/+} and *Lep*^{+/+}/*HSL*^{-/-}. Although the precise mechanism should be elucidated, cAMP/cAMP-dependent protein kinase A (PKA) pathway might be involved. Glucagon-like peptide (GLP)-1 increases cell survival via cAMP-dependent stimulation of cAMP response element binding protein (CREB) activity, and subsequent enhancement of the insulin receptor substrate (IRS)-2 regulated pathway [27]. The decreased feeding behavior in *Lep*^{ob/ob}/*HSL*^{-/-} might reduce the serum Glucagon-like peptide (GLP)-1 levels.

The long-term exposure to FFA leads to disruption of GSIS [28], and genetic deletion of HSL decreases plasma FFA levels mobilized from adipose tissue in both *Lep*^{+/+} and *Lep*^{ob/ob} background. Since deficiency of HSL raises numerous changes caused from phenotype of adipose tissue or the other tissues, tissue-specific genetic deletion will be required to further determine the precise role of HSL on pancreatic β -cells.

It was reported that lipids generated by acylglycerol hydrolysis play a critical role in insulin secretion using a chemical inhibition of β -cell lipolysis [4]. However, it still remained to be determined whether the lipase(s) responsible for the regulation of insulin secretion would be HSL or the other lipase(s). It was reported recently that adipose triglyceride lipase is another promising candidate [29]. In the present study, we demonstrated that HSL is at least one of the key molecules involved in the insulin secretory machinery.

In summary, we clarified the role of HSL in pancreatic β -cells by the co-ablation of leptin gene. It was demonstrated that the deficiency of HSL in *Lep*^{ob/ob} mice causes impaired insulin secretion and reduces the islet mass due to the defective cell proliferation. These findings provide the basis for understanding the pathophysiology of obesity and diabetes.

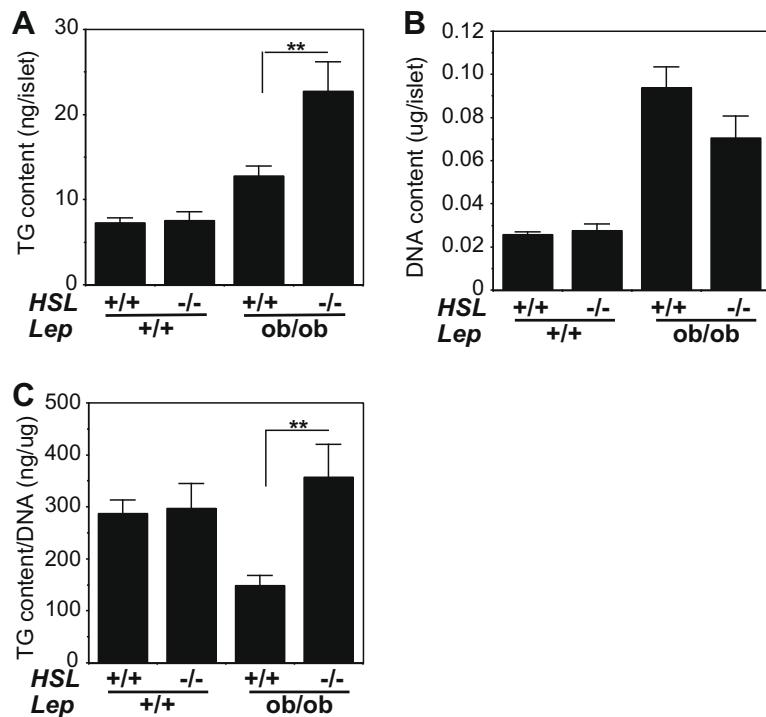


Fig. 3. Excessive triglyceride accumulation was observed in *Lep*^{ob/ob}/*HSL*^{-/-} islets ($n = 9$ –11). (A) Lipids were extracted from ~100 islets/each mouse by the chloroform-methanol method. (B) Lipid extracted islets were dissolved in phosphate buffered saline by sonication, and the DNA content was measured by fluorescent method. (C) TG content of islets was normalized with respect to DNA content. Each value represents the mean \pm SE. **Denotes significance at $P < 0.01$, vs *HSL*^{+/+} mice in the same *Lep* background determined by the Student's *t*-test.

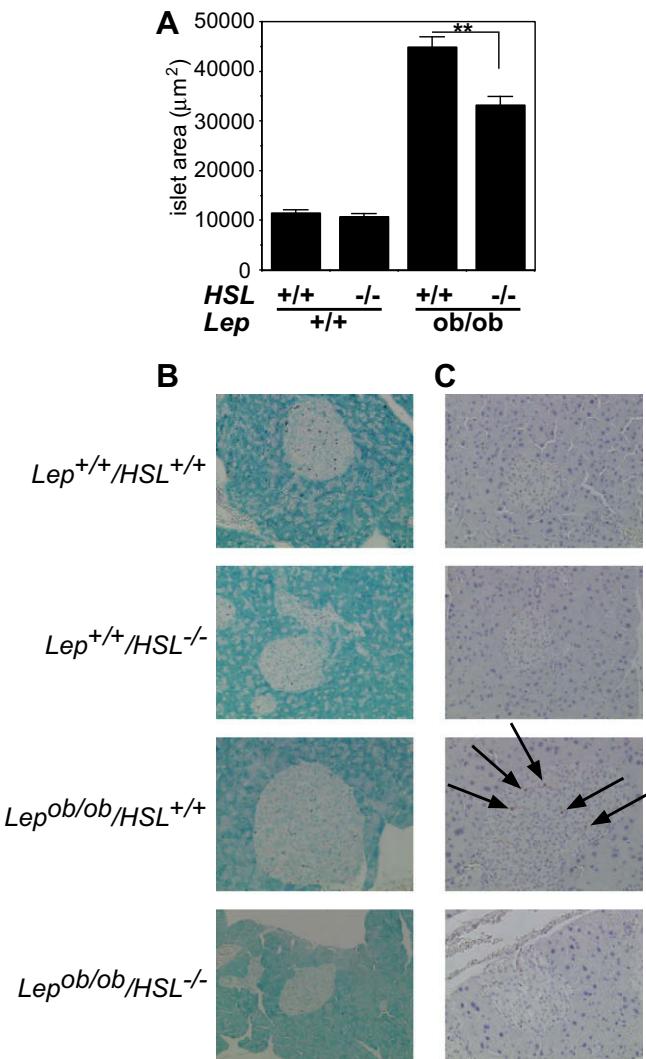


Fig. 4. The deficiency of HSL reduced the pancreatic islet mass of *Lep*^{ob/ob} mice. (A) Islet mass was quantified using NIH image. At least 50 islets from each pancreas were quantified and total number of islets scanned was 195–260 for each genotype ($n = 3$). Each value represents the mean \pm SE. **Denotes significance at $P < 0.01$, respectively, vs *HSL*^{+/+} mice in the same *Lep* background determined by the Student's *t*-test. (B, C) Immunohistochemical analysis of islets. (B) TUNEL staining and (C) BrdU labeling. Arrows point to the labeled cells (C).

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