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Original Article

Ginsenoside Rb1 increases insulin sensitivity through suppressing 11β-hydroxysteroid dehydrogenase type I

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Abstract: Ginsenoside Rb1 (GRb1) is a major component of ginseng, which has been shown to ameliorate hyperglycemia in rodents and in humans with undetermined mechanisms. Here, we analyzed the molecular mechanisms by which GRb1 reduces the insulin resistance in high-fat diet (HFD)-induced mouse model for type 2 diabetes (T2D). HFD was applied for 4 weeks to induce T2D in mice, after which GRb1 was administrated and the effects on the fasting blood glucose, glucose tolerance and insulin sensitivity were analyzed. We found that HFD increased fasting blood glucose, glucose tolerance and reduced insulin sensitivity, which were all ameliorated by GRb1. GRb1 seemed to reduce the levels of 11β-Hydroxysteroid dehydrogenase type I (11β-HSD1) in liver and adipose tissue, to exert its anti-diabetes effects. Overexpression of 11β-HSD1 completely abolished the effects of GRb1 on HFD-induced increases in fasting blood glucose and glucose tolerance, and decreases in insulin sensitivity. Together, our data suggest that GRb1 may increase insulin sensitivity through suppressing 11β-HSD1 in treatment of T2D.

Keywords: Ginsenoside Rb1 (GRb1), type 2 diabetes (T2D), 11β-Hydroxysteroid dehydrogenase type I (11β-HSD1), insulin resistance

Introduction

Failure to maintain a tightly regulated blood glucose level results in a metabolic disease, called diabetes [1]. Among all diabetes cases, the majority is type 2 diabetes (T2D), in which the insulin loses its potent effects in regulating blood glucose, mostly by impaired insulin production and secretion and induction of insulin resistance in peripheral tissue [2-4]. The prevalence of T2D has risen enormously over the last decades and the final solution is still unavailable despite great advances that have been made in the past.

Glucocorticoid, as an antagonist for insulin, regulates multiple metabolic processes including central obesity, insulin resistance and glucose intolerance. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) catalyses conversion of inactive cortisone to active cortisol in adipose tissue to enhance local effects of glucocorticoid and thus to trigger glucocorticoid-related obesity and T2D [5-8]. Previous studies have shown that 11β-HSD-1 activity is significantly increased in adipose tissue of obese animals and obese humans [9-12]. Mice that overexpress 11β-HSD1 showed increases in local glucocorticoid levels, and features of the obesity-associated metabolic disorders, e.g. dyslipidemia, insulin resistance, and glucose intolerance [13]. On the other hand, 11β-HSD1-knockout mice produced reduced glucocorticoid in adipose tissues and exhibited enhanced insulin sensitivity. Thus, 11β-HSD1 levels are associated with obesity, glucose intolerance and insulin resistance.

Ginseng is widely used herb in many medical approaches and has been used in treating T2D [14]. Ginseng has been shown to ameliorate hyperglycemia in rodents [15-18] and in humans [19, 20]. Ginsenoside Rb1 (GRb1) is a major component of ginseng, has been found to have therapeutic effects in treating obese and diabetes [21-23]. However, the molecular mechanisms underlying the effects of Ginseng and GRb1 in such occasions are unknown. Here, we analyzed the molecular mechanisms by which GRb1 reduces the insulin resistance in high-fat diet (HFD)-induced mouse model for type 2 diabetes (T2D).

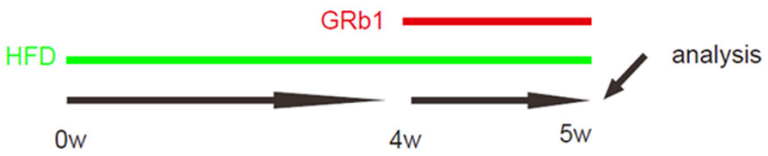

Figure 1. Schematic of the experiments. Mice of 12 weeks of age were randomly divided into two groups: the normal-diet group (ND) and the high-fat diet (HFD) group. After 4 weeks of ND or HFD, the mice of HFD group were administrated with GRb1 or control saline of same frequency and same volume, for one week before analysis.

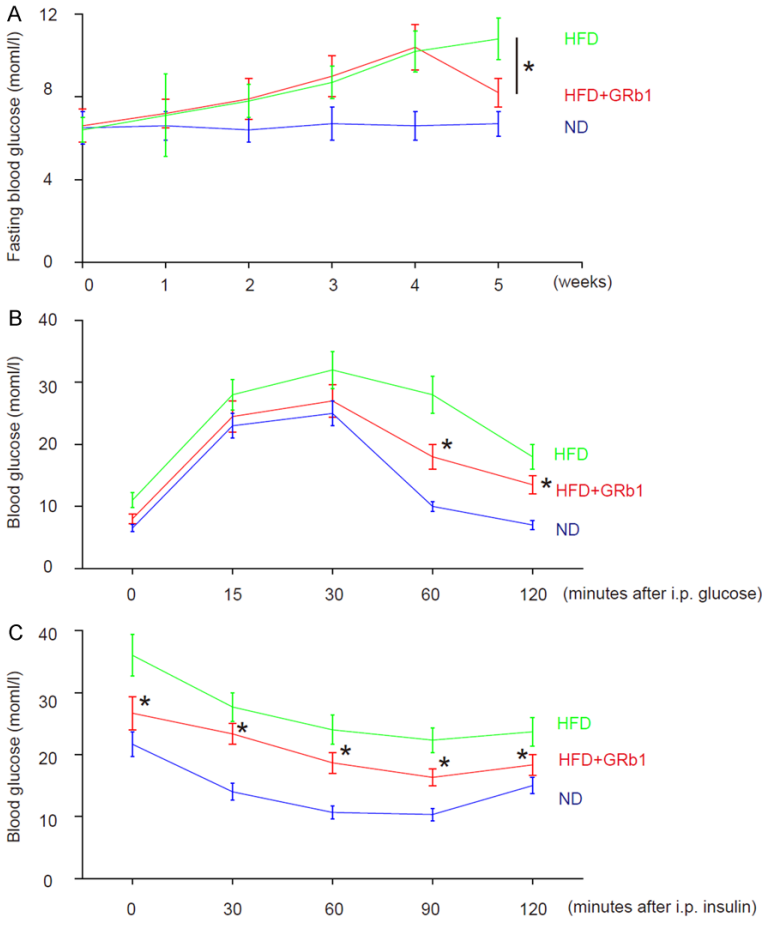

Figure 2. GRb1 attenuates HFD-induced increases in fasting blood glucose, glucose tolerance and HFD-induced reduction in insulin sensitivity. A. Fasting blood glucose. B. Glucose tolerance test. C. Insulin tolerance test. *P<0.05. N=10.

Materials and methods

Mouse treatment

All animal experiments were performed according to the Institutional guidance for Care and Use of Laboratory Animals, and the experimental protocols were approved by the Ethics Committee for Experimental Research from the First Hospital affiliated to Jinzhou Medical University. Female C57BL/C mice of 12 weeks of age were purchased from the National Resource Center of Model Mice (Nanjing, China). Mice were housed in Pathogen-free environment. The animals were randomly divided into two groups: the normal-diet group (ND) and the high-fat diet (HFD) group. After 4 weeks of ND or HFD, the mice of HFD group were i.p. administrated with 10 mg/kg GRb1 (Weikeqi Bioscience, China) every other day for 1 week. The control mice received saline of same frequency and same volume. AAV injection was through tail vein and the dose is 10^8 viral particles in 100 μl.

Generation of AAVs

AAV-CMV-11β-HSD1-2A-GFP (simplified as AAV-11β-HSD1) and AAV-GFP were prepared as has been previously described [24]. Briefly, a pAAV-CMV-GFP plasmid (Clontech, Mountain View, CA, USA), a packaging plasmid carrying the serotype 8 rep and cap genes and a helper plasmid carrying the adenovirus helper functions (Applied Viromics, LLC. Fremont, CA, USA) were co-transfected the HEK293 cells for generating AAVs, using Lipofectamine 2000 reagent (Invitrogen). The virus purification was done with CsCl density centrifugation and titration was determined by a quantitative densitometric dot-blot assay.

Physiological assessments

Fasting blood glucose levels were measured using an Accu-Chek glucose meter (Roche, Indianapolis, IN, USA). For intraperitoneal glu-

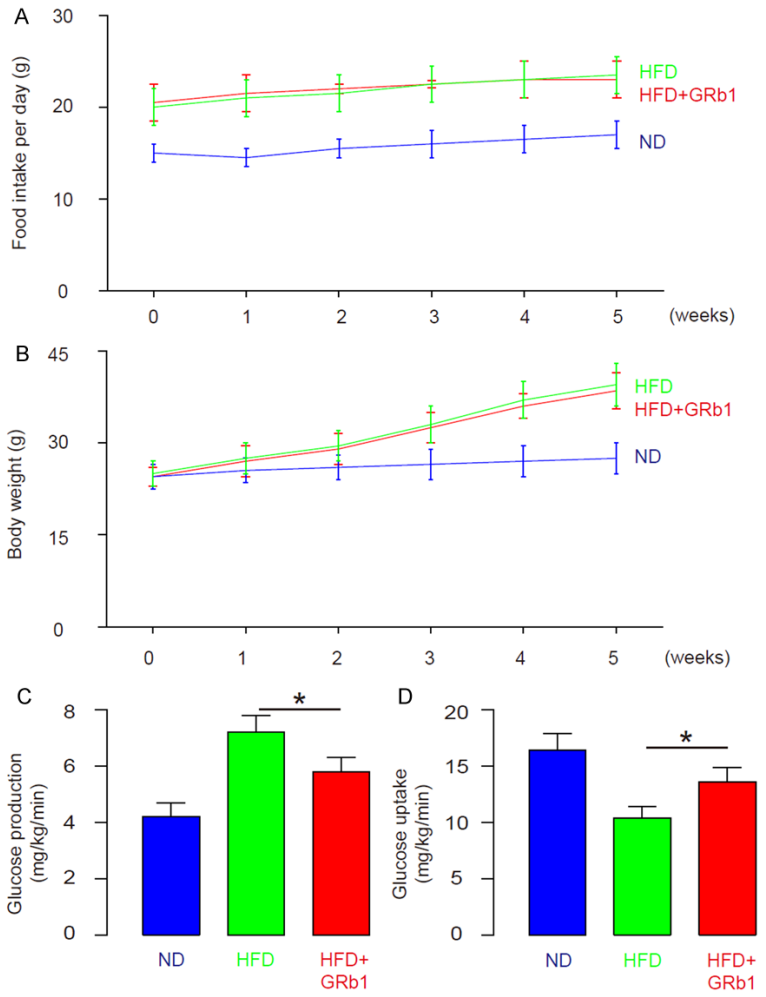

Figure 3. GRb1 reduces glucose production and increases glucose uptake. A. Food intake. B. Body weight. C. Glucose production in liver. D. Glucose uptake in skeletal muscle. *P<0.05. N=10.

cose tolerance test (IPGTT), mice were fasted for 16 hours and injected with glucose (2 g/kg, i.p.). Blood glucose levels were measured at 15, 30, 60 and 120 minutes after injection. For insulin tolerance test, mice were fasted for 16 hours and injected with insulin (0.5 unit/kg, i.p.). Blood glucose levels were measured at 30, 60, 90 and 120 minutes after injection. For analysis of the rate of glycogen synthesis, rate of glycogen synthesis, 100 mg liver tissue was rinsed with cold phosphate-buffered saline (PBS) and solubilized by incubating with 1 mol/l KOH (0.5 ml) at 80°C for 30 min. After centrifugation, the supernatant was transferred to a new tube, 95% ethanol (550 μl) was added and the pellet was washed with ice-cold 95% ethanol and re-suspended with 300 μl ddH_2O. Part of the solution (150 μl) was transferred in duplicate to scintillation vials, and ^{14}C-radioactivity was counted. The activity per tissue mass was then normalized to the integral of the plasma specific activity of the 2-[14C] DG over the time of exposure, to yield a rate of incorporation into glycogen. For glucose up-take analysis, 100 mg skeletal mu-scle was added into 250 μl of 1N NaOH and incubated at 80°C for 30 min to digest the tissues. The solutions were neutralized with 250 μl of 1N HCl, and then centrifuged at 14,000 rpm for 15 min. An aliquot of the supernatant was precipitated by $HClO_4$ and quantified by liquid scintillation counting to determine total tissue values (dpm) for the sum of 2-[^{14}C] DG and 2-[^{14}C] deoxyglucose phosphate (2-[^{14}C] DGP). Another aliquot was deproteinized with 0.3N zinc sulfate ($ZnSO_4$) and 0.3N barium hydroxide [$Ba(OH)_2$] to precipitate 2-[^{14}C] DG6P and quantify 2-[^{14}C] DG in the supernatant. The value for the 2-[^{14}C] DG in the supernatant (dpm) was subtracted from the total tissue 2-[^{14}C] DG and 2-[^{14}C] DGP (dpm) to calculate the glucose uptake rate as indicated by the skeletal muscle 2-[^{14}C] DGP accumulation.

Immunohistochemistry

Mouse tissue were dissected out and fixed with 4% paraformaldehyde (Sigma-Aldrich) for 6 hours, and then cyro-protected in 30% sucrose for 24 hours. H&E staining was performed.

Western blot

Western blot was performed as previous described [24]. Primary antibodies for Western Blot are anti-11β-HSD1 and anti-β-actin (Cell Signaling, San Jose, CA, USA). Secondary antibody is HRP-conjugated anti-rabbit (Jackson

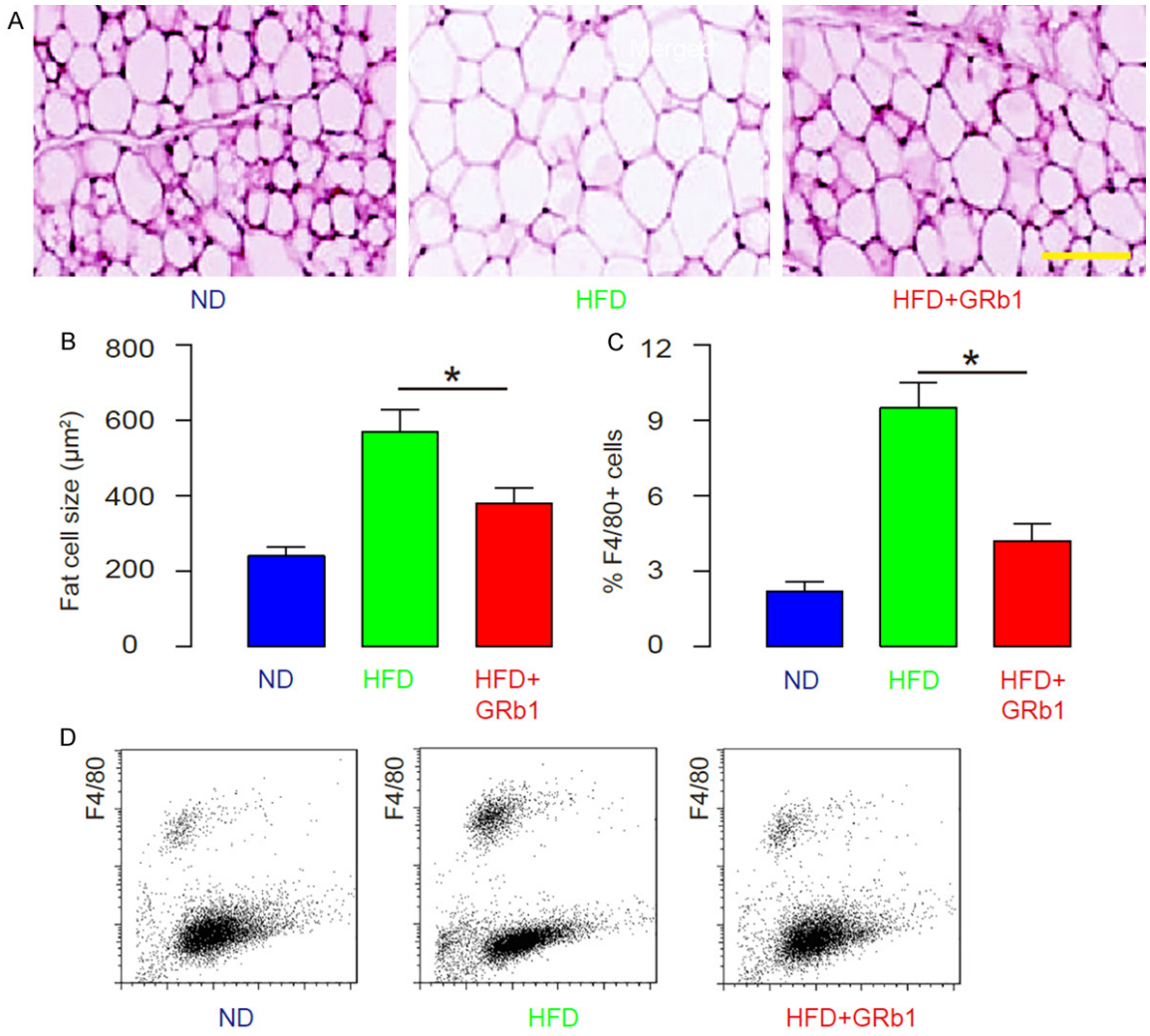

Figure 4. GRb1 reduces visceral fat index in HFD-treated mice. (A, B) The visceral fat cell size, by representative images (A), and by quantification (B). (C, D) The F4/80+ cells in the fat tissue analyzed by flow cytometry, shown by quantification (C), and by representative flow charts (D). *P<0.05. N=10. Scale bar is 50 μm.

ImmunoResearch Labs, West Grove, PA, USA). Images shown in the figure were representative from 5 repeats.

Flow cytometry

F4/80-based cell analysis was performed by flow cytometry, using dissociated adipose tissue cells that were labeled with PEcy7-conjugated anti-F4/80 antibodies (Becton-Dickinson Biosciences, San Jose, CA, USA). Flow cytometry was performed using a FACSAria (Becton-Dickinson Bioscien-ces) flow cytometer. Negative controls were applied to remove background noise and to confirm positive cells. Data were analyzed and quantified using Flowjo software (Flowjo LLC, Ashland, OR, USA).

Statistics

All values are depicted as mean ± standard deviation and are considered significant if P<0.05. All data were statistically analysed using one-way ANOVA with a Bonferroni correction, followed by Fisher's Exact Test to compare 2 groups.

Results

Schematic of the experiments

Mice of 12 weeks of age were randomly divided into two groups: the normal-diet group (ND) and the highfat diet (HFD) group. After 4 weeks of ND or HFD, the mice of HFD group were administrated with GRb1 or control saline of same

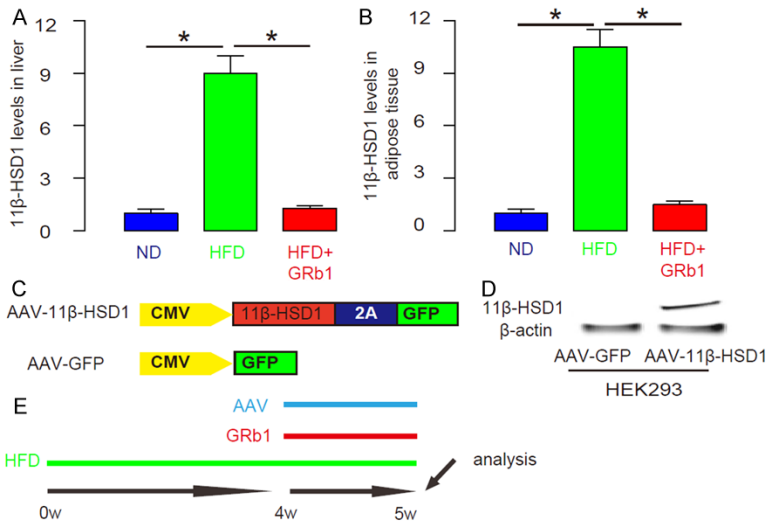

Figure 5. GRb1 decreases 11β-HSD1 levels in HFD-treated mice. (A, B) The Western blot for 11β-HSD1 levels in liver (A) and in adipose tissue (B) in HFD-treated mice. (C) AAV-CMV-Pax4-2A-GFP was generated to carry 11β-HSD1 and GFP, AAV-CMV-Pax4-2A-GFP (simplified as AAV-11β-HSD1). AAV-CMV-GFP was used as a control (simplified as AAV-GFP). (D) Western blot for AAV-11β-HSD1. (E) Schematic of the experiment. A single viral infusion through tail vein were given at the time of GRb1. *P<0.05. N=10.

frequency and same volume, for one week before analysis (**Figure 1**).

GRb1 attenuates HFD-induced increases in fasting blood glucose, glucose tolerance and HFD-induced reduction in insulin sensitivity

We found that HFD increased fasting blood glucose (**Figure 2A**), glucose tolerance (**Figure 2B**) and reduced insulin sensitivity (**Figure 2C**), which were all ameliorated by GRb1 (**Figure 2A-C**). Thus, GRb1 attenuates HFD-induced increases in fasting blood glucose, glucose tolerance and HFD-induced reduction in insulin sensitivity.

GRb1 reduces glucose production and increases glucose uptake

We found that GRb1 did not alter either food intake (**Figure 3A**) or body weight (**Figure 3B**), but significantly reduced glucose production in liver (**Figure 3C**), and significantly increased glucose uptake in skeletal muscle (**Figure 3D**). These data suggest that GRb1 may enhance insulin sensitivity in HFD-treated mice.

GRb1 reduces visceral fat index in HFD-treated mice

We found that HFD significantly increased the visceral fat cell size (**Figure 4A**, **4B**), and significantly increased the F4/80+ cells in the fat tissue (**Figure 4C**, **4D**). However, GRb1 treatment significantly reduced the visceral fat cell size in HFD-treated mice (**Figure 4A**, **4B**), and significantly decreased the F4/80+ cells in the fat tissue in HFD-treated mice (**Figure 4C**, **4D**). Thus, GRb1 reduces visceral fat index in HFD-treated mice.

GRb1 decreases 11β-HSD1 levels in HFD-treated mice

We found that 11β-HSD1 levels significantly increased in liver (**Figure 5A**) and in adipose tissue (**Figure 5B**) in HFD-treated mice. In order to examine the role of 11β-HSD1 in GRb1-treated HFD-mice, we generated AAV vectors that carry 11β-HSD1 and GFP, AAV-CMV-Pax4-2A-GFP (simplified as AAV-11β-HSD1). AAV-CMV-GFP (simplified as AAV-GFP) was used as a control (**Figure 5C**). The quality of AAV-11β-HSD1 was confirmed in Western blot (**Figure 5D**). Then, a single viral infusion through tail vein were given at the time of GRb1 (**Figure 5E**).

Overexpression of 11β-HSD1 levels abolishes the effects of GRb1 on HFD-induced increases in fasting blood glucose, glucose tolerance and HFD-induced reduction in insulin sensitivity

We found that injection of AAV-11β-HSD1 completely abolished the effects of GRb1 on HFD-induced increases in fasting blood glucose (**Figure 6A**), glucose tolerance (**Figure 6B**) and HFD-induced decreases in insulin sensitivity (**Figure 6C**).

Overexpression of 11β-HSD1 levels abolishes the effects of GRb1 on glucose production, glucose uptake and visceral fat index

We found that injection of AAV-11β-HSD1 completely abolished the effects of GRb1 on HFD-induced increases in glucose production in liver (**Figure 7A**), on HFD-induced reduction in glucose uptake in skeletal muscle (**Figure 7B**), on

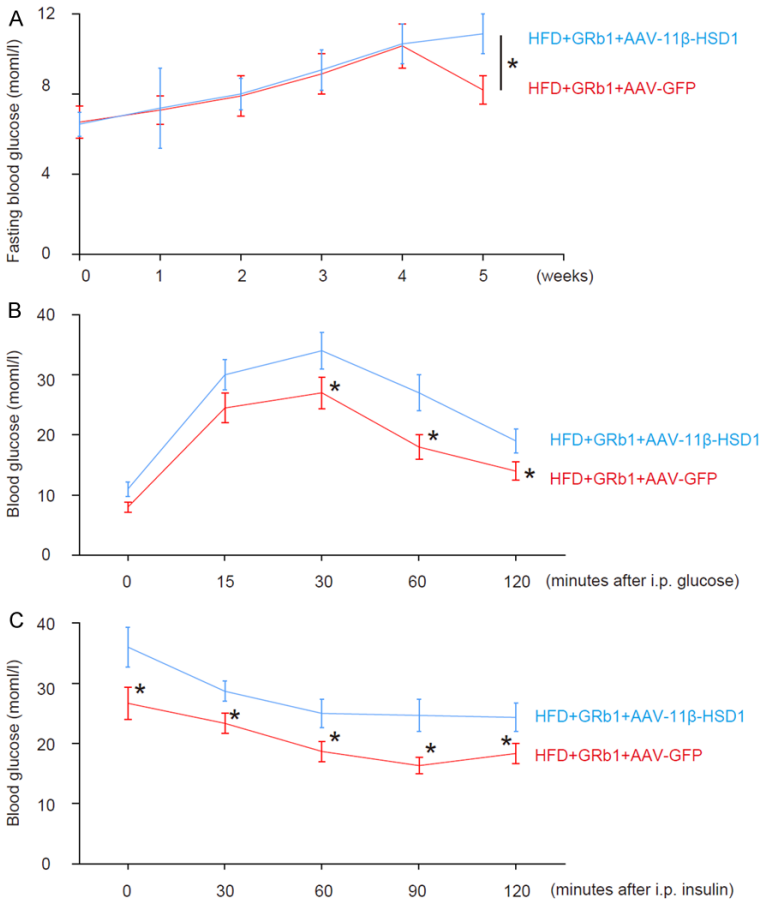

Figure 6. Overexpression of 11β-HSD1 levels abolishes the effects of GRb1 on HFD-induced increases in fasting blood glucose, glucose tolerance and HFD-induced reduction in insulin sensitivity. A. Fasting blood glucose. B. Glucose tolerance test. C. Insulin tolerance test. *P<0.05. N=10.

visceral fat cell size (**Figure 7C**, **7D**), and on F4/80+ cells in adipose tissue (**Figure 7E**, **7F**). Together, our data suggest that GRb1 may increase insulin sensitivity through suppressing 11β-HSD1.

Discussion

Some Chinese traditional medicine have been shown to have pronounced therapeutic effects on a number of different diseases. However, the mixture manner of these Chinese traditional medicine prevents a precise determination of the molecular mechanisms underlying their therapeutic effects. GRb1 is a purified and defined item from Ginseng with reported effects in protection and treatment of obesity, insulin resistance and T2D. Here, we showed that in a well-defined T2D model, HFD, application of GRb1 attenuated the increases in fasting blood glucose, glucose intolerance and insulin insensitivity. Since the alterations of glucose synthesis in liver, glucose update in muscle and visceral fat index by HFD were all attenuated by GRb1 without changes in either food intake or body weight, the insulin resistance induced by HFD appeared to be ameliorated.

Among all the factors that are associated with pathogenesis of obesity, insulin resistance and T2D, 11β-HSD1 was specifically detected as an altered one by HFD and by GRb1. 11β-HSD1 triggers the activation of glucocorticoid to antagonize the insulin effects. Previous studies have shown that 11β-HSD1 suppresses the insulin sensitivity by downregulating the expression of insulin-signaling pathway associated proteins, e.g. PI3K, Akt and GLUT4. Thus, the effects of GRb1 may affect insulin resistance status directly through modification of cortisol by 11β-HSD1. These hypothesis were confirmed in a loss-of-function experiment, in which forced expression of 11β-HSD1 diminished the effects of GRb1 in this model. The infection of the mouse tissue by AAV will turn on the transgene within 2 days [25-27], which is sufficient to examine the effects of the transgene.

In future, the direct regulation of 11β-HSD1 by GRb1 should be analyzed in vitro to fully understand the molecular signaling pathways that are involved. Here, we provide compelling evidence to demonstrate a role of GRb1 in treating obesity, insulin resistance and T2D. These results may be fundamental for generating GRb1-based therapy for T2D.

Acknowledgements

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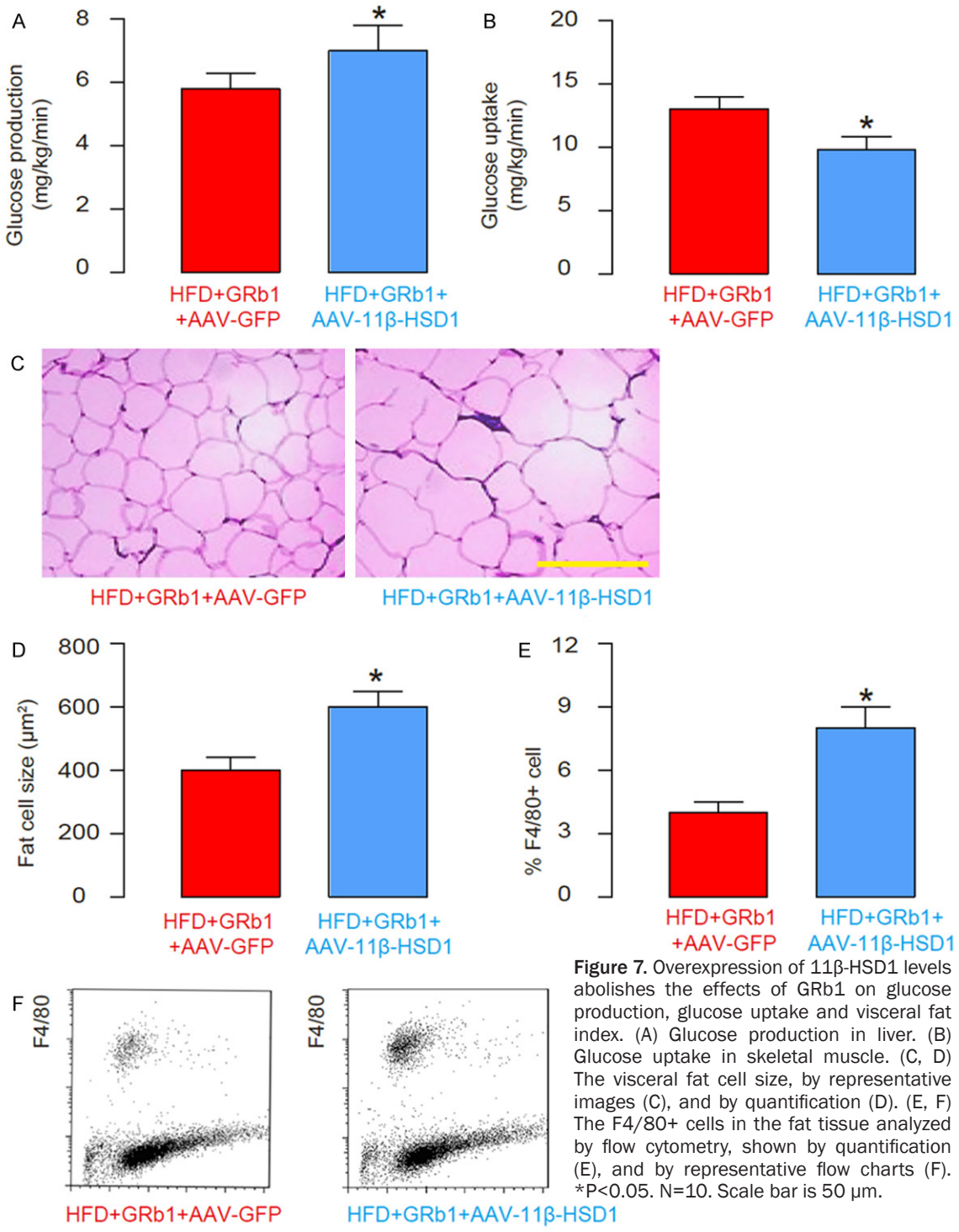

Figure 7. Overexpression of 11β-HSD1 levels abolishes the effects of GRb1 on glucose production, glucose uptake and visceral fat index. (A) Glucose production in liver. (B) Glucose uptake in skeletal muscle. (C, D) The visceral fat cell size, by representative images (C), and by quantification (D). (E, F) The F4/80+ cells in the fat tissue analyzed by flow cytometry, shown by quantification (E), and by representative flow charts (F). *P<0.05. N=10. Scale bar is 50 μm.

Disclosure of conflict of interest

None.

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