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Original Article

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Differential Diabetogenic Effect of Pitavastatin and Rosuvastatin, *in vitro* and *in vivo*

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Aim: Most statins increase the risk of new-onset diabetes. Unlike other statins, pitavastatin is reported to exert neutral effects on serum glucose level, but the precise mechanism is unknown.

Methods: Eight-week-old male C57BL/6J mice ($n=26$) were fed high-fat diet (HFD, 45% fat) with 0.01% placebo, rosuvastatin, or pitavastatin for 12 weeks. Cultured HepG2, C2C12, and 3T3-L1 cells and visceral adipocytes from HFD-fed mice were treated with vehicle or 10 μM statins for 24 h. The effects of pitavastatin and rosuvastatin on intracellular insulin signaling and glucose transporter 4 (GLUT4) translocation were evaluated.

Results: After 12 weeks, the fasting blood glucose level was significantly lower in pitavastatin-treated group than in rosuvastatin-treated group (115.2 ± 7.0 versus 137.4 ± 22.3 mg/dL, $p=0.024$). Insulin tolerance significantly improved in pitavastatin-treated group as compared with rosuvastatin-treated group, and no significant difference was observed in glucose tolerance. Although plasma adiponectin and insulin levels were not different between the two statin treatment groups, the insulin-induced protein kinase B phosphorylation was weakly attenuated in pitavastatin-treated adipocytes than in rosuvastatin-treated adipocytes. Furthermore, minor attenuation in insulin-induced GLUT4 translocation to the plasma membrane of adipocytes was observed in pitavastatin-treated group.

Conclusion: Pitavastatin showed lower diabetogenic effects than rosuvastatin in mice that may be mediated by minor attenuations in insulin signaling in adipocytes.

Key words: Pitavastatin, Rosuvastatin, Diabetes, Adipocyte, Insulin resistance, Insulin signaling

Background

Statins are 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors that are proven to be beneficial and widely used for the prevention of cardiovascular diseases[1-4]. However, several clinical trials have shown that statins may increase the risk of new-onset diabetes mellitus (NOD)[5-7]. Although statins cause significant diabetogenic effects, the precise mechanism is incompletely known. Several possible mechanisms have been hypothesized, including the effect of statins on the survival of pancreatic beta cells, reduction in insulin secretion, enhancement in hepatic glucose production, increased insulin resistance through the disruption of intracellular insulin signaling, and disturbance of glucose uptake in the peripheral tissues such as muscle and fat[8-12]. Whether these effects are common to all statins is questionable.

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In comparison with other statins, pitavastatin exhibited neutral effects on serum glucose and NOD risk[7, 13]. In addition, pitavastatin does not appear to increase glucose or glycated hemoglobin level[13-15]. A recent meta-analysis revealed the absence of any association between pitavastatin and increased risk of NOD[16].

There are several hypothetical mechanisms explaining the action of pitavastatin on glucose metabolism. The pharmacological profile and effects of pitavastatin on adiponectin were thought to be related to its influence on glucose metabolism[17]. How pitavastatin affects glucose metabolism is, however, still unclear. Here, we investigated the effects of pitavastatin on glucose metabolism by focusing on insulin sensitivity, glucose tolerance, intracellular insulin signaling, and adiponectin levels in diet-induced obese mice and under *in vitro* conditions.

Materials and Methods

Animals

Six-week-old male C57BL/6J mice were housed under standard conditions (21±2℃, 60±10% humidity, 12 h light/dark cycle) with *ad libitum* access to food and water. The mice were fed a high-fat diet (HFD, including 45% fat, $n=6$), HFD with rosuvastatin (0.01%, $n=10$), or HFD with pitavastatin (0.01%, $n=10$) for 12 weeks beginning from 8 weeks of age. Food intake, fasting blood glucose, and body weight were measured twice a week (in the evening after 8 h of fasting). After 12 weeks, the mice were euthanized under anesthesia and the blood samples were collected by cardiac puncture. The liver and epididymal fat tissues were isolated from each mouse and weighed. All animal procedures were performed in accordance with the guidelines from the National Institutes of Health and pre-approved by the animal care and use committee at the Yonsei University, College of Medicine (2014-0304).

Oral Glucose Tolerance Test (OGTT) and Insulin Tolerance Test (ITT)

OGTT and ITT were performed after the mice had received the test diet for 11 weeks at 19 weeks of age. After 8 h of fasting, the baseline blood glucose level was measured by tail vein puncture. For OGTT, a solution of 40% glucose (2 g/kg body weight) was administered by oral gavage. After glucose administration, blood samples were collected from the tail vein at 15, 30, 60, 90, and 120 min. Blood glucose levels were measured using Accu-Chek Performa glucometer (Boehringer-Mannheim, Indianapolis, IN, USA).

For ITT, the mice were fasted for 8 h under non-anesthetized conditions. Insulin-R (I9278, Sigma-Aldrich, St. Louis, MO, USA) was intraperitoneally injected (0.75 U/kg body weight) and blood samples from the tail vein were collected at 15, 30, 60, 90, and 120 min after insulin injection. Glucose levels were evaluated with Accu-Chek Performa glucometer (Boehringer-Mannheim, Indianapolis, IN, USA).

Fasting Plasma Insulin and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR)

The blood samples collected after 8 h of fasting were used for the quantification of plasma insulin level with an enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's recommendations (EZRMI-13K, EMD Millipore Corporation, St. Charles, MO, USA). HOMA-IR was estimated from fasting glucose and insulin as follows:

HOMA-IR = fasting insulin (μU/mL) × fasting glucose (mg/dL)/405 [18].

Measurement of Plasma Total Adiponectin and High Molecular Weight (HMW) Adiponectin Levels

The blood samples were collected in microcentrifuge tubes and centrifuged to obtain the serum. Serum adiponectin and HMW adiponectin levels were measured using mouse ELISA kits (47-ADPMS-D01, ALPCO, Salem, NH, USA).

Cell Culture and Drug Treatment

The hepatocellular carcinoma cell line, HepG2, was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin (SV30010) in a 5% CO_2 incubator at 37℃. The immortalized murine myoblast cell line, C2C12, was cultivated in Eagle's Minimum Essential Medium supplemented with 2 mM L-glutamine, 10% dialyzed FBS, and antibiotics[19]. Preadipocyte 3T3-L1 cells were cultured in a differentiation medium[20]. Rosuvastatin (SML1264, Sigma-Aldrich, St. Louis, MO, USA) and pitavastatin (CAS 147526-32-7, Santa Cruz Biotechnology, Santa Cruz, CA, USA) were dissolved in dimethyl sulfoxide before dilution in the culture medium. In all experiments, the final statin and dimethyl sulfoxide concentrations were 10 μM and 0.1%, respectively.

Western Blot Analysis for Protein Kinase B (Akt) and Phosphorylated Akt

Primary adipocytes from the mouse visceral adipose tissue and 3T3-L1, HepG2, and C2C12 cells were pretreated with 10 μM pitavastatin or rosuvastatin for 24 h, serum-starved for 4 h with or without statins, and stimulated with 100 nM insulin for 5 to 15 min. Cell lysates were obtained from each treat-

Table 1. Characteristics of mice fed HFD, HFD with rosuvastatin, and HFD with pitavastatin

	HFD ($n=6$)	HFD + Rosuvastatin ($n=10$)	HFD + Pitavastatin ($n=10$)	p value
Baseline (8 weeks)				
Body weight	25.7 ± 1.8	23.4 ± 1.4*	24.2 ± 1.7	0.028
At the end of treatment (20 weeks)				
Body weight (g)	39.2 ± 6.5	34.8 ± 4.6	34.2 ± 3.0	0.110
Weight gain (g)	13.4 ± 5.1	11.4 ± 4.3	10.0 ± 3.6	0.323
Liver (g)	1.4 ± 0.3	1.3 ± 0.3	1.2 ± 0.2	0.433
Epididymal fat (g)	2.0 ± 0.5	1.7 ± 0.8	1.7 ± 0.5	0.670

Data are expressed as the mean ± SD and compared by one-way ANOVA, Dunnett's post-test. *$p<0.05$ versus HFD group. HFD; high fat diet.

ment group.

Equal amounts of proteins were electrophoresed on sodium dodecyl sulfate polyacrylamide gels and the separated protein bands were transferred onto polyvinylidene fluoride membranes. After blocking, the membranes were incubated with phospho-Akt serine 473 (#9271, Cell Signaling Technology, Denver, MA, USA), phospho-Akt threonine 308 (#9275), and pan-Akt (#9272) antibodies, followed by treatment with horseradish peroxidase-conjugated anti-rabbit IgG (sc-2030, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The blots were developed using an enhanced chemiluminescence detection kit. Equal loading was verified by re-probing the blot with β-actin (A5441, Sigma, St Louis, MO, USA) antibody.

2-Deoxyglucose (2-DG) Uptake and Glucose Transporter 4 (GLUT4) Translocation Staining

2-DG uptake into 3T3-L1 cells was measured using Glucose Uptake Colorimetric Assay Kit (ab136955, Abcam Inc. Cambridge, MA, USA) according to the manufacturer's protocol. Measurements were performed in triplicates and the average value was recorded.

GLUT4 staining was conducted as previously described[21]. After stimulation with 100 nM insulin for 30 min, the cells were fixed on coverslips with 4% paraformaldehyde and permeabilized with 0.25% Triton X-100. GLUT4 level was detected by incubation with a polyclonal primary antibody (ab654, Abcam Inc. Cambridge, MA, USA) using standard procedures. After washing with phosphate-buffered saline (PBS), the binding of the primary antibody was detected with an Alexa-Fluor 488-conjugated secondary antibody (A21206, Invitrogen, Carlsbad, CA, USA). Coverslips were mounted with ProLong Gold antifade medium (P36934, Invitrogen, Carlsbad, CA, USA) and imaged using LSM700 laser confocal microscope (Zeiss, Jena, Germany).

Statistical Analysis

All data are presented as the mean ± standard deviation (SD). Statistical analyses were performed with t-test, analysis of variance (ANOVA), or repeated measures ANOVA with Dunnett's t-test for multiple comparisons as appropriate. A value of $p<0.05$ was considered significant. Analysis was performed using SPSS Statistics version 23.0 (IBM Co., Somers, NY, USA).

Results

Biochemical Characteristics of Mice

The characteristics of mice from different groups are described in Table 1. Baseline body weight was the lowest among the mice from rosuvastatin group. At the end of the treatment (after 12 weeks), no difference was observed in the body weight and degree of weight gain between all groups. Furthermore, the mean liver weight and fat mass were not significantly different among the mice from the three groups (all $p>0.05$).

No difference in body weight gain was observed between pitavastatin and rosuvastatin treatment groups over the 12-week study period (Fig. 1a). No statistical difference was reported in food intake among the three groups (Fig. 1b). However, fasting blood glucose level was significantly lower in pitavastatin group than in rosuvastatin group after 12 weeks of treatment (115.2 ± 7.0 mg/dL in pitavastatin group versus 137.4 ± 22.3 mg/dL in rosuvastatin group, $p=0.024$, Fig. 1c).

Effects of Pitavastatin and Rosuvastatin on Glucose and Insulin Tolerance

The results of OGTT showed that the mice from pitavastatin group exhibited a significant improvement in glucose tolerance as compared with those from control and rosuvastatin groups at 0, 30, and

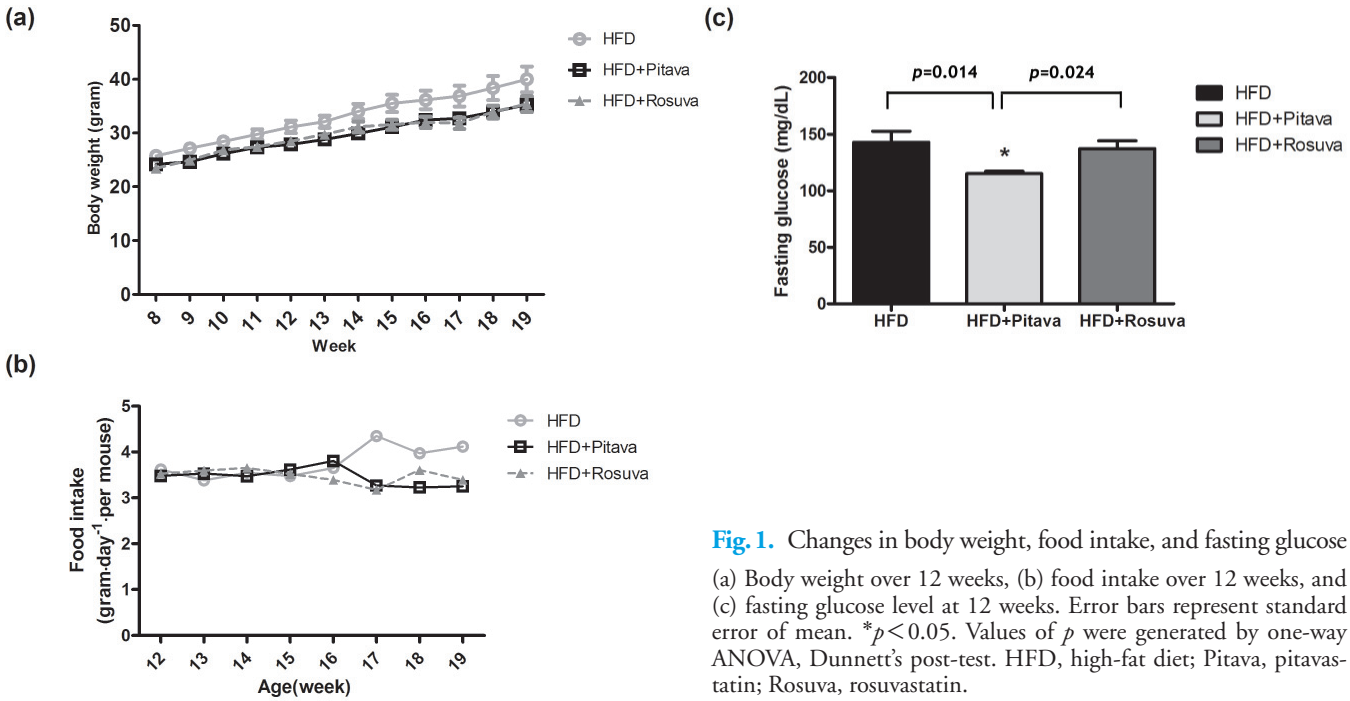

Fig. 1. Changes in body weight, food intake, and fasting glucose

(a) Body weight over 12 weeks, (b) food intake over 12 weeks, and (c) fasting glucose level at 12 weeks. Error bars represent standard error of mean. *$p<0.05$. Values of p were generated by one-way ANOVA, Dunnett's post-test. HFD, high-fat diet; Pitava, pitavastatin; Rosuva, rosuvastatin.

120 min after glucose administration (all $p<0.05$, **Fig. 2a**). The area under the curve (AUC) value of OGTT was lower for pitavastatin group than for HFD and rosuvastatin groups, but no statistically significant difference was reported (**Fig. 2b**, $p=0.080$).

Insulin tolerance improved in pitavastatin group as compared with the control group at 15 and 60 min after insulin administration (all $p<0.05$, **Fig. 2c**). Furthermore, the AUC value of ITT significantly reduced in both pitavastatin and rosuvastatin groups (**Fig. 2d**, $p=0.008$ for pitavastatin and $p=0.013$ for rosuvastatin).

Effects of Pitavastatin and Rosuvastatin on Plasma Insulin and Insulin Resistance

The mean plasma insulin concentration was 11.2, 9.2, and 8.8 μU/mL in the control, rosuvastatin, and pitavastatin groups, respectively (**Fig. 3a**). No significant difference was reported between the three groups, although the plasma insulin level tended to be lower in pitavastatin group than in other groups ($p=0.138$). Insulin resistance, as indicated by HOMA-IR value, was the lowest in pitavastatin group ($p=0.048$, **Fig. 3b**).

Effects of Pitavastatin and Rosuvastatin on Plasma Adiponectin and HMW Adiponectin Levels

The plasma adiponectin level increased in pitavastatin group as compared to the control and rosuvastatin groups, although no significant difference was reported (21,779.9 ± 3,317.5, 17,409.2 ± 3,453.3, and 20,139.6 ± 2,070.8 ng/dL in pitavastatin, HFD, and rosuvastatin groups, respectively; $p=0.105$) (**Fig. 4a**). No significant differences were observed in HMW adiponectin levels between the three groups ($p=0.653$, **Fig. 4b**).

Effects of Pitavastatin and Rosuvastatin on Insulin Signaling

To analyze the effect of pitavastatin on insulin response in the liver, we evaluated the phosphorylation level of Akt after 15 min from stimulation of HepG2 cells with insulin. No significant difference was observed in the level of Akt phosphorylation at serine 473 and threonine 308 in HepG2 cells from the three groups (all $p>0.05$ versus control + insulin group, **Fig. 5a**).

Intracellular insulin signaling level in the muscle was analyzed using C2C12 cells (**Fig. 5b**). After 15 min of insulin administration, no significant difference was observed in Akt phosphorylation level at serine 473 and threonine 308 among the three insulin-stimulated groups (all $p>0.05$ versus control + insulin group).

To confirm the role of statins in the adipose tissue, intracellular insulin signaling was investigated in 3T3-L1 adipocytes (after differentiation). After insulin stimulation, Akt phosphorylation level at serine 473 and threonine 308 showed attenuated pattern in rosuvastatin-treated cells as compared with control cells (**Fig. 5c**). Akt phosphorylation at serine 473 showed a decreasing trend in rosuvastatin group as compared

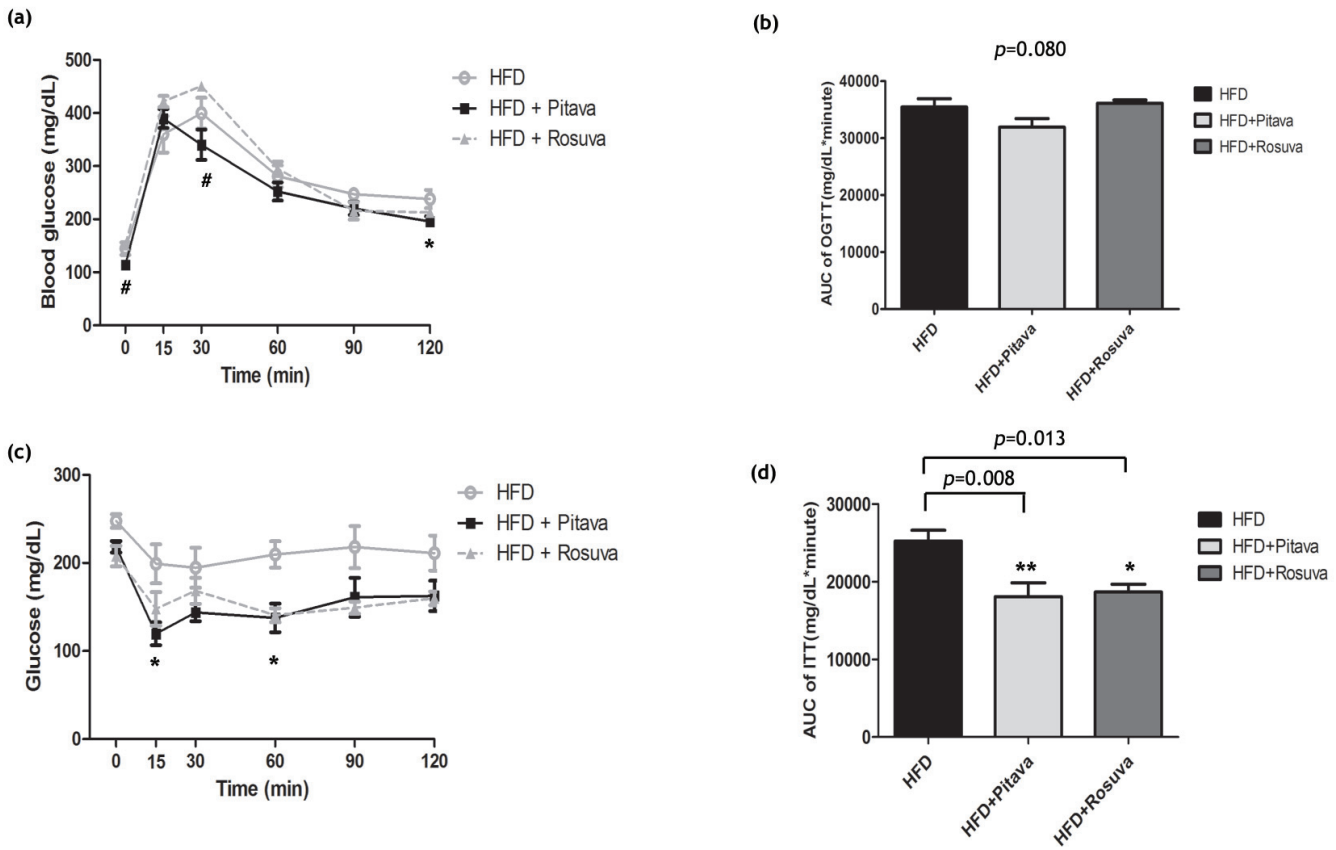

Fig. 2. Systemic glucose metabolism evaluated with oral glucose tolerance test and insulin tolerance test

(a) Oral glucose tolerance test, (b) area under the curve of glucose tolerance test, (c) insulin tolerance test, (d) area under the curve of insulin tolerance test. Error bars represent standard error of mean.
$^{*}p<0.05$, $^{**}p<0.01$ versus corresponding HFD value. $^{\#}p<0.05$, pitavastatin versus rosuvastatin.
p values were generated by ANOVA, Dunnett's post-test.
HFD, high-fat diet; OGTT, oral glucose tolerance test; AUC, area under the curve; Pitava; pitavastatin; Rosuva, rosuvastatin.

with control group ($p=0.054$). Furthermore, Akt phosphorylation at threonine 308 significantly reduced in the presence of rosuvastatin as compared with the control group ($p=0.002$). Akt phosphorylation in pitavastatin group was not significantly different from that observed in the control group (all $p>0.05$).

The above experiments were also conducted in an *ex vivo* model with primary mouse adipocytes obtained from the visceral fat tissue of HFD-fed mice (**Supplementary Fig. 1**). The level of Akt phosphorylation at threonine 308 was significantly attenuated in rosuvastatin group as compared with control group after 15 min of insulin administration ($p=0.049$).

Attenuation of Akt Phosphorylation Inhibits Glucose Uptake and GLUT4 Translocation

We conducted an experiment to confirm whether the attenuation of Akt phosphorylation by statins leads to a decrease in glucose uptake. In 3T3-L1 cells, the reduction in 2-DG uptake was observed in rosuvastatin group (versus control + insulin, $p=0.042$, **Fig. 6a**).

GLUT4 staining showed a significant increase in the translocation of GLUT4 to the membrane after insulin administration in pitavastatin group than in rosuvastatin group (**Fig. 6b**). These observations confirm that the difference in glucose uptake between adipocytes may arise from the variations in GLUT4 translocation to the plasma membrane.

Discussion

In the present study, we evaluated the effects of pitavastatin on glucose metabolism in HFD-fed obese mice and several cell lines and compared them with the effects of a widely used drug, rosuvastatin. In comparison with rosuvastatin treatment group, pitavas-

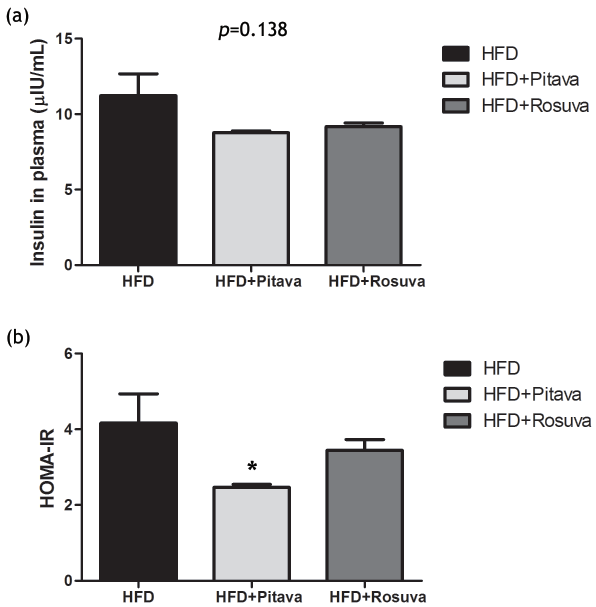

Fig. 3. Plasma insulin levels and HOMA-IR in different treatment groups

(a) Insulin level, (b) HOMA-IR. Error bars represent standard error of mean. *$p<0.05$ versus corresponding HFD value. All p values were generated by ANOVA. HFD, high-fat diet; Pitava, pitavastatin; Rosuva, rosuvastatin; HOMA-IR, homeostatic model assessment of insulin resistance.

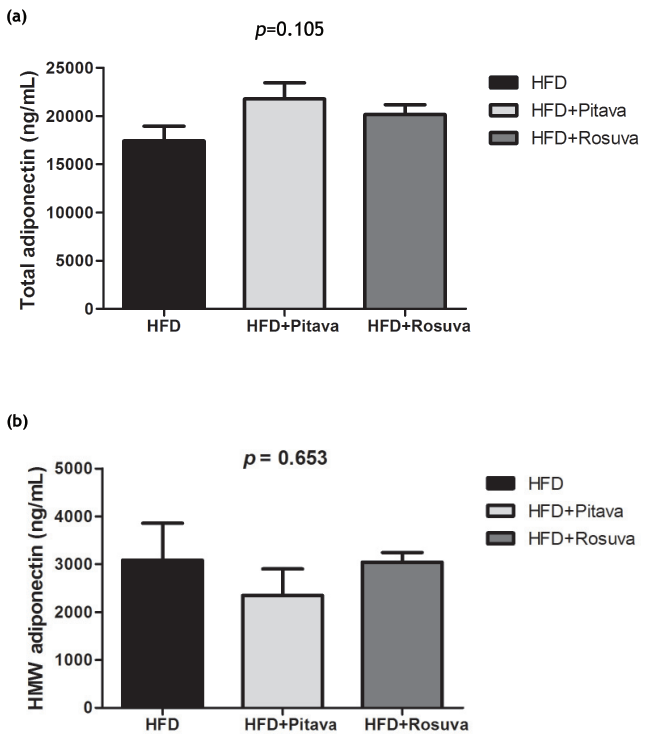

Fig. 4. Plasma adiponectin and high molecular weight (HMW) adiponectin level in different treatment groups

(a) Total adiponectin, (b) HMW adiponectin. Error bars represent standard error of mean. *P* values were generated by ANOVA. HFD, high-fat diet; Pitava, pitavastatin; Rosuva, rosuvastatin.

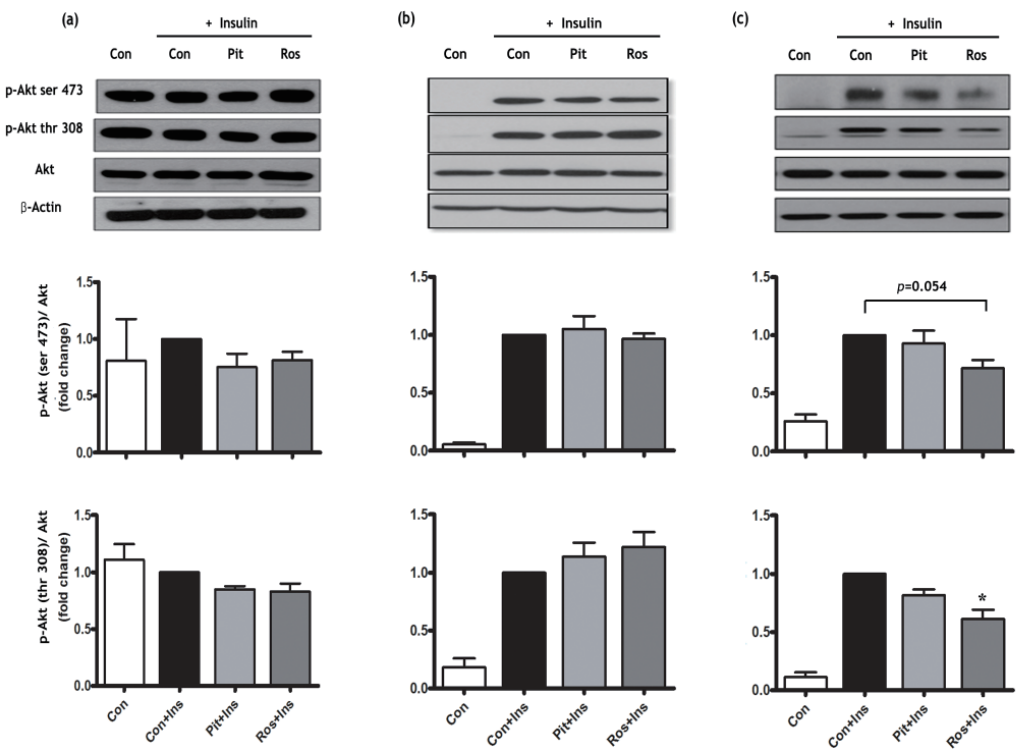

Fig. 5. Statins differentially alter the insulin-induced phosphorylation of Akt in cultured cells

Cells were treated with 10 μM of statins for 24 h, followed by insulin stimulation for 5 to 15 min. (a) HepG2 cells, (b) C2C12 cells, (c) 3T3-L1 cells. Representative western blots are shown above the plots of densitometric analysis of p-Akt/Akt ratio in relation to insulin-stimulated control cells (means ± SE, $n=3$-4). Dunnett's *t*-test was used to compare the values of statin-treated groups and insulin-stimulated control group. *$p<0.05$ versus control + insulin. Con, control; Pit, pitavastatin; Ros, rosuvastatin; Ins, insulin; SE, standard error.

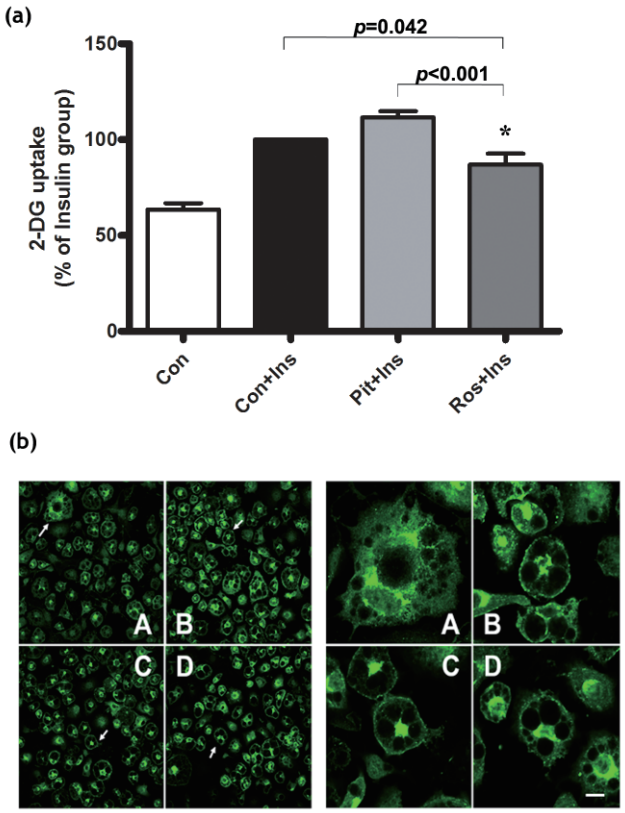

Fig. 6. Statins differentially alter the insulin-induced 2-DG uptake and GLUT4 translocation in cultured adipocytes

(a) Comparison of 2-DG uptake. Data represent means ± SE of three independent experiments and are expressed as fold changes in glucose uptake relative to that with control + insulin group. Dunnett's *t*-test was used to compare the values of statin-treated groups and insulin-stimulated control group. $^{*}p<0.05$, $^{**}p<0.01$ (b) GLUT4 staining, A, Con; B, Con + Ins; C, Pit + Ins; D, Ros + Ins. GFP is shown in green (Scale bar: 10 μm (micrometer)).
GLUT4, glucose transporter 4; 2-DG, 2-deoxyglucose; Con, control; Pit, pitavastatin; Ros, rosuvastatin; Ins, insulin; SE, standard error; GFP, green fluorescent protein.

tatin treatment group showed lower fasting glucose level without any significant difference in food intake and weight gain. Considering the result of OGTT and ITT, the main reason underlying the difference in glucose metabolism between the two statin groups was thought to be the variation in insulin resistance. We could confirm that this effect may be mediated by the reduced attenuation in insulin signaling in adipocytes.

Insulin-induced glucose uptake in the peripheral adipose tissue is crucial for the reduction in blood glucose concentrations, and the defect in this process is one of the major causes of diabetes mellitus. The affinity of pitavastatin toward hepatic LDL receptors is lower than that of other statins[22]. As a consequence, pitavastatin exhibits reduced hepatic absorption rate and increased systemic bioavailability (≥ 51%) and it may strongly influence the systemic glucose uptake in peripheral cells such as myocytes and adipocytes[17]. However, in the present study, we found that the use of pitavastatin did not significantly affect the process of insulin signaling in the muscle or adipose tissue. This may serve as one of the reasons underlying the neutral effects of pitavastatin on glucose metabolism.

Insulin signaling in the peripheral adipose tissue is mediated by the binding of insulin to the cell surface receptors, resulting in the phosphorylation of insulin receptor substrates at specific tyrosine residues and activation of phosphatidylinositol 3-kinase and its downstream target Akt. Akt and the downstream targets have key roles in insulin sensitivity and normal glucose tolerance[23, 24]. Phosphoinositide-3,4,5-trisphosphate produced by phosphatidylinositol 3-kinase[25] binds to the domain of Akt, allowing for its translocation to the cell membrane, followed by phosphorylation and activation within the catalytic domain T-loop (threonine 308) and the carboxyl terminal hydrophobic domain (serine 473) and the subsequent activation of many downstream targets[26, 27]. In the next step, it redistributes GLUT4 from the intracellular storage to the plasma membrane. Therefore, the reduction in Akt activation in the adipose tissue may potentially increase insulin resistance and the risk of diabetes. In addition, the differences in the expression of GLUT4 in adipocytes were thought to be one of the reasons for the observed differences in the effects of statins on glucose metabolism[28]. However, in the present study, the attenuation of Akt phosphorylation in adipocytes was lower in the presence of pitavastatin than with rosuvastatin treatment. In addition, the difference in Akt attenuation between pitavastatin and rosuvastatin groups led to the variation in GLUT4 translocation to the plasma membrane and 2-DG uptake in adipocytes.

Previous studies have reported attenuated insulin signaling and decreased GLUT4 translocation in adipocytes when using atorvastatin[29] or lovastatin[30]. In this study, rosuvastatin also showed a similar effect, while pitavastatin did not show such patterns. Previous studies in humans have shown increased insulin resistance following treatment with several statins, including rosuvastatin[31]. However, consistent with present study, pitavastatin was shown to exert no effect on blood glucose control and whole body insulin sensitivity[32]. The mechanisms by which some statins may exert diabetogenic effects currently remain unclear. Several recent meta-analyses have suggested that a differential effect on incidence of diabetes[33] or insulin sensitivity[34] may exist for different statins in human. Statins comprise many subtypes based on structural

differences, resulting in different pharmacokinetics and efficacy. This structural difference between statins could lead to a difference in efficiency in transportation into the cell[35] and differential diabetogenic effect.

Clinical data have shown that pitavastatin increases plasma adiponectin and HMW adiponectin levels[36-39], which are potent insulin sensitizers[40, 41]. This phenomenon is often presented as one of the important reasons underlying the lower risk of diabetes with pitavastatin than with other statins. However, in the present study, no statistically significant difference was observed in the serum levels of adiponectin and HMW adiponectin in different treatment groups. The present findings provide evidence that pitavastatin exerts neutral effects on insulin signaling and resistance without changing the levels of adiponectin. In previous studies, the possibility that the use of pitavastatin may directly act on fat tissue via prevention of adipocyte hypertrophy[42] and anti-adipogenic action[43] was reported. We could not proceed with a related experiment in the current study, and instead, we measured the size of adipocyte (visceral) using a similar design model from a study conducted in our laboratory. The results also showed that the cell size in adipose tissue of pitavastatin treatment group was significantly decreased compared to the high fat control or rosuvastatin treatment group (all $p<0.001$ by ANOVA, **Supplementary Fig. 2**). Likewise, the result of this study was consistent with those of previously reported studies in that the difference in the effect of pitavastatin on glucose metabolism, as compared with other statins, is mainly associated with the variation in its effect on adipocytes.

In vitro experiments revealed the lack of any significant difference in insulin signaling in hepatocytes and muscle cells from pitavastatin and rosuvastatin treatment groups. Only adipocytes from pitavastatin treatment group were less affected during insulin signaling and were less involved in insulin resistance compared to those from rosuvastatin treatment group. Recent studies on statins have reported increased insulin resistance in adipose tissue. Henriksbo *et al.* reported that statin activates caspase-1/IL-1β inflammasome responses and impairs endocrine control of adipocyte lipogenesis[44]. However, further studies will be needed to determine why these effects are specific for adipocyte.

In the current study, insulin responsive secretion evoked by increased insulin resistance was not observed in rosuvastatin group. Increased insulin resistance with persistent high fat diets could decrease the function of insulin secretion in rosuvastatin group. Also, hepatic gluconeogenesis plays an important role in determining fasting insulin level, as well as the function of beta cell secretion. In this study, there was no significant difference in the effect of statin on liver. As a result, this may lead to similar fasting insulin levels. However, the direct effect of statins on the pancreas and the function of insulin secretion was not fully investigated in this study, and further studies will be needed to complement these factors.

The present study also had several other limitations. The change in serum cholesterol level was not measured. Therefore, whether the experiment was performed under the conditions that produce similar effects on cholesterol remains a concern. In addition, the effect of pitavastatin was compared only with rosuvastatin; we could not confirm whether similar results could be reproducible with other statins. Despite these limitations, the study has several merits. We investigated the effects of pitavastatin on glucose metabolism via intracellular insulin signaling, insulin resistance, and glucose clearance in HFD-induced obese mice and several cell lines. Furthermore, we demonstrated that pitavastatin is less involved in the increased peripheral insulin resistance through the decreased attenuation of insulin-stimulated intracellular signaling via Akt phosphorylation and GLUT4 translocation.

Conclusion

Pitavastatin treatment reduced fasting blood glucose and insulin resistance than rosuvastatin treatment. This observation was associated with the improvement in peripheral insulin resistance, especially in the adipose tissue, via intracellular Akt activation and GLUT4 translocation. Pitavastatin may serve as a useful drug for the treatment of dyslipidemia in patients with a high risk of insulin resistance or hyperglycemia.

List of Abbreviations

GLUT4, glucose transporter 4; NOD, new-onset diabetes mellitus; HFD, high-fat diet; OGTT, oral glucose tolerance test; ITT, insulin tolerance test; HOMA-IR, homeostatic model assessment of insulin resistance; HMW, high molecular weight; Akt, protein kinase B; 2-DG, 2-deoxyglucose; AUC, area under the curve.

Declarations

Ethics approval and consent to participate: All animal procedures were performed in accordance with the guidelines from the National Institutes of Health

and pre-approved by the animal care and use committee at the Yonsei University, College of Medicine (2014-0304).

Consent for Publication

Not applicable.

Availability of Data and Material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Authors' Contributions

Y. Cho, E.Y. Choe, H. Lee and E. S. Kang designed the experiment, interpreted the experimental results, and wrote the manuscript. H. Park, H.J. Wang, R-H Kim, and Y. Kim designed and conducted the experiments. H. Lee and E. S. Kang revised the manuscript. All authors contributed to manuscript preparations & discussion and approved the final manuscript.

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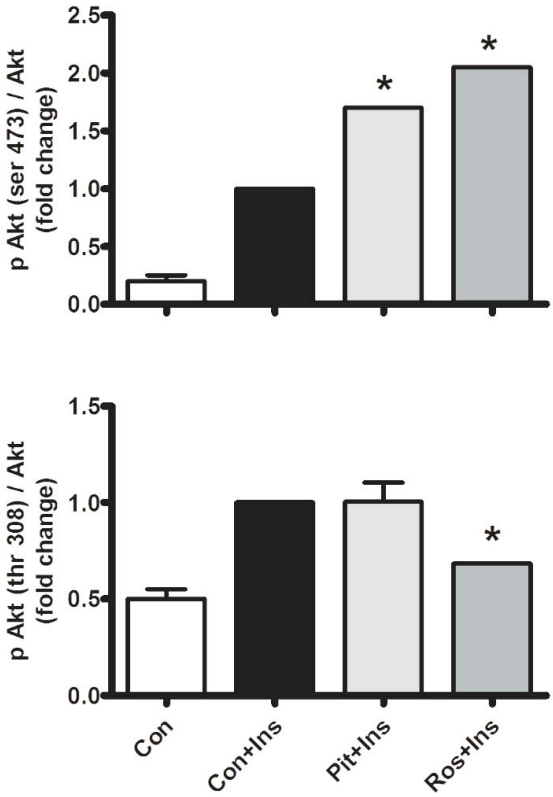
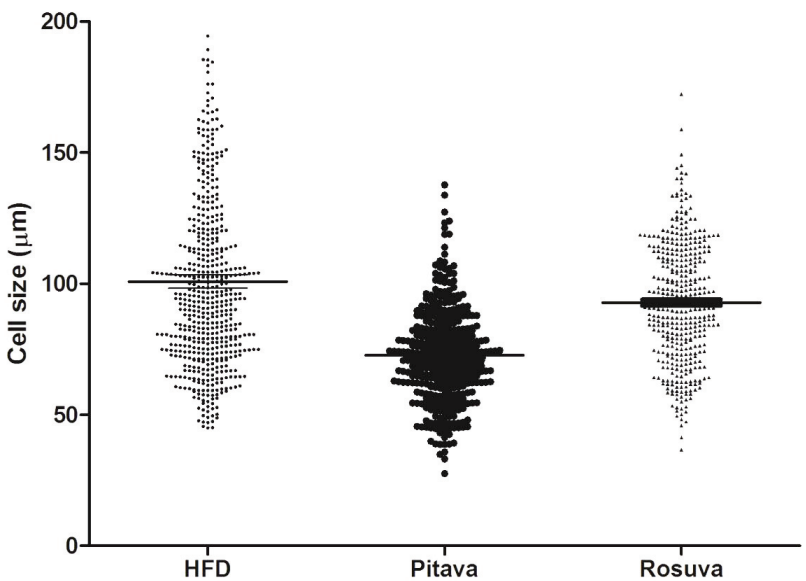


Supplementary Fig. 1. Statins differentially alter the insulin-induced phosphorylation of Akt in cultured cells

Primary adipocytes from the visceral fat tissue were treated with 10 μM of statins for 24 h, followed by stimulation with insulin for 5 to 15 min. (a) Visceral adipocytes from mice fed with high-fat diet.
Representative western blots are shown above the plots of densitometric analysis of p-Akt/Akt ratio in relation to insulin-stimulated control cells (means ± SE, $n=2$). Dunnett's t-test was used to compare the values between statin-treated groups and insulin-stimulated control group. $^{*}p<0.05$ versus control + insulin. Con, control; Pit, pitavastatin; Ros, rosuvastatin; Ins, insulin.



Supplementary Fig. 2. Statins differentially alter the size of adipocytes

Mice were fed a high-fat diet (HFD, including 45% fat, $n=4$), HFD with rosuvastatin (0.01%, $n=3$), or HFD with pitavastatin (0.01%, $n=3$) for 15 weeks, starting from 5 weeks of age .
Graph data were compiled to show the effects of pitavastatin and rosuvastatin on the diameter of adipocytes in a total of 1,200 adipocytes ($n=120$ per animal). Markedly increased size of adipocytes were observed in both high fat control group and rosuvastatin treated group with high fat diet, but the sizes were less increased in pitavastatin treated group with high fat diet mouse model (all $p<0.001$ by ANOVA test).
HFD, high fat diet control; Pitava, pitavastatin treated with high fat diet; Rosuva, rosuvastatin treated with high fat diet.