

Modulation of sphingosine-1-phosphate and apolipoprotein M levels in the plasma, liver and kidneys in streptozotocin-induced diabetic mice

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Keywords

Apolipoprotein M, Sphingosine-1-phosphate, Streptozotocin-induced diabetes mice

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ABSTRACT

Aims/Introduction: Sphingosine-1-phosphate (S1P), a multifunctional bioactive lipid mediator, is involved in various diseases. Apolipoprotein M (ApoM) carries S1P on high-density lipoprotein and modulates S1P metabolism to increase the total S1P mass in the body. Both S1P and ApoM are involved in diabetes.

Materials and Methods: The present study examined the modulation of S1P and ApoM levels in the plasma, liver and kidneys in streptozotocin-induced diabetes (STZ) mice, and the effects of insulin on the S1P and ApoM levels in the plasma and liver in STZ mice and normal mice. We also examined the effects of insulin and glucose on the ApoM levels in HepG2 cells.

Results: In STZ mice, both the plasma S1P and ApoM levels were higher than those in control mice. The hepatic S1P and ApoM contents were also elevated. The hepatic S1P and ApoM contents were reduced by insulin treatment, whereas high-dose insulin decreased the plasma S1P and ApoM levels. In mice without streptozotocin treatment, the administration of insulin decreased the plasma S1P and ApoM levels, and the hepatic content of ApoM, whereas the hepatic level of S1P was not altered. Treatment with insulin and incubation under a low glucose level decreased the ApoM levels in HepG2 cells. Regarding the kidney, the renal levels of S1P and ApoM were increased in STZ mice, and insulin treatment partially restored this increment.

Conclusions: In STZ mice, the levels of S1P and ApoM in the plasma, liver, and kidneys were increased. Insulin treatment somehow reversed this modulation in STZ mice.

INTRODUCTION

The prevalence of diabetes is now increasing worldwide. β -Cell death and insufficient β -cell function are the pathogenetic causes of type 1 and type 2 diabetes, respectively^{1–3}. The physiological regulators of β -cell function, however, have not yet been completely elucidated.

Sphingosine-1-phosphate (S1P), a bioactive lipid mediator, is known to be involved in various diseases, such as cardiovascular

diseases, cancers and congenital disorders^{4,5}. Among these diseases, the involvement of S1P in diabetes, especially β -cell function, has been proposed based on *in vitro* experiments. For example, sphingosine kinase (SphK), a limiting enzyme for S1P synthesis, reportedly has significant roles in both β -cell function and survival^{6–8}. Therefore, S1P might be deeply involved in the pathogenesis of diabetes.

Regarding the modulation of S1P metabolism in a diabetic state, diabetes reportedly influences the plasma S1P level and the S1P contents in several tissues; the plasma S1P level was higher in streptozotocin-induced diabetic rats and Akita mice,

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whereas the S1P content in the heart of Ins2Akita mice was also increased⁹. Furthermore, insulin treatment restored the elevated S1P level in Akita mice⁹, suggesting that insulin plays an important role in controlling the S1P level, although the underlying mechanism has not yet been elucidated.

Regarding the dynamics of S1P, approximately 65% of circulating S1P is carried on apolipoprotein M (ApoM)¹⁰. ApoM is one of the apolipoproteins riding on high-density lipoprotein, and its expression is confined to the liver and kidneys¹¹. We recently showed that ApoM is not a mere carrier of S1P, but a potent modulator of S1P in both the plasma and liver, with ApoM increasing S1P, at least in part, by retarding the degradation of S1P¹². Despite this crucial influence on S1P, the ApoM levels were reported to be relatively low in some diabetic animals; in alloxan-induced diabetic mice, in which the glucose levels are elevated as a result of the impairment of β -cell function by alloxan, the plasma ApoM level and the messenger ribonucleic acid levels of ApoM in the liver and kidney were reduced, but were partially restored by insulin treatment¹³.

This disagreement between the modulation of S1P and ApoM in diabetic mice suggests that a link between S1P and ApoM might not exist in diabetic mice; however, no study has measured the S1P and ApoM levels simultaneously or even in the same animal models. Furthermore, in contrast to the results of animal experiments, ApoM expression was reportedly reduced by treatment with insulin in HepG2 cells¹⁴. Considering the important roles of S1P in the biology of β -cells, the elucidation of the modulation of ApoM and S1P levels in an insulin-insufficient state is an important task from the aspect of the possible cross-talk between β -cells and the liver, which is the major source of circulating ApoM.

In the present study, we investigated the modulation of S1P and ApoM levels in the plasma, liver, and kidney of STZ mice simultaneously to elucidate whether the modulation of ApoM might be correlated with the dynamics of S1P in diabetic mice.

MATERIALS AND METHODS

Animal Experiments

C57BL/6 mice were purchased from CLEA Japan (Tokyo, Japan). Ten-week-old male C57BL/6 mice were intraperitoneally injected with streptozotocin (150 mg/kg bodyweight), whereas control mice were injected with the same amount of saline. On the third day, the blood glucose levels under *ad libitum* feeding were measured, and mice with blood glucose levels of over 300 mg/dL were deemed as streptozotocin mice (STZ). Eight weeks after the administration, the mice were subjected to 6-h fast, and a plasma sample was collected from the postorbicular capillary; liver and kidney samples were obtained after perfusion with ice-chilled phosphate-buffered saline (PBS) through a needle inserted into the left ventricle. The basic characteristics of the STZ and control mice are described in Table 1.

To investigate the effects of insulin on the S1P and ApoM levels in STZ mice, STZ mice (STZ mice [group 2] in Table 1) were injected intraperitoneally with Humulin R (Ely Lilly,

Table 1 | Characteristics of mice analyzed

	Vehicle	STZ group 1 [‡]	STZ group 2 [‡]
FBG (mg/dL)	155.6 ± 12.2	541.0 ± 10.9 [†]	533.8 ± 20.4 [†]
BW (g)	31.4 ± 0.9	22.5 ± 0.7 [†]	21.9 ± 1.6 [†]
Liver weight (g)	1.59 ± 0.05	1.68 ± 0.02	1.54 ± 0.09
EP fat weight (g)	0.88 ± 0.08	0.09 ± 0.02 [†]	0.06 ± 0.02 [†]

[†] $P < 0.01$ vs vehicle; [‡]Streptozotocin-induced diabetes (STZ) group 1 was subjected to analyses with the vehicle, while STZ group 2 was injected with insulin (Humulin R at a dose of 0.75 U/kg of bodyweight); the analyses were carried out 2 h later. BW, bodyweight; EP, epididymal; FBG, fasting blood glucose.

Indianapolis, IN, USA) at a dose of 0.75 IU/kg bodyweight. To investigate the effects of various doses and types of insulin, 12-week-old male STZ mice prepared as aforementioned were injected intraperitoneally with Humulin R at a dose of 2 IU/kg bodyweight, or injected subcutaneously with Humulin N (Ely Lilly) at a dose of 80 IU/kg bodyweight. Plasma samples were then collected at 2 and 16 h after the insulin administration. To investigate the effects of insulin and glucose in mice without STZ treatment, 11-week-old male mice were treated with 0.5 IU/kg bodyweight of Humulin R, 2 g/kg bodyweight of glucose, or PBS (as a control). Plasma samples were collected before injection, and plasma, liver and kidney samples were collected at 2 h after injection. To investigate the effects of insulin in ApoM-overexpressing mice, we infected 10-week-old male mice with human ApoM-coding adenovirus through the tail vein at a dose of 2.5×10^8 pfu/g of bodyweight, as described previously¹², then on the fifth day, the effects of insulin on the plasma S1P levels were examined with 0.5 IU/kg bodyweight of Humulin R, as aforementioned. All the animal experiments were carried out in accordance with the guidelines for animal care at the Graduate School of Medicine, The University of Tokyo, Tokyo, Japan, and were approved by the animal committee of the University of Tokyo.

Cell Experiments

HepG2 cells were obtained from American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich Co., St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Gibco BRL, Eggenstein, Germany) and 1% penicillin/streptomycin (Gibco BRL). At approximately 70% confluence, the cells were washed twice with PBS and replaced for FBS-free DMEM containing various doses of glucose and human insulin solution (Sigma-Aldrich Co.), and after 8 h the media and cellular components were collected.

Sphingosine Kinase Activity Assay

We measured the sphingosine kinase (SphK) activity levels as described previously¹⁵, with some modification. We homogenized liver tissues in 20 mmol/L Tris-HCl (pH 7.4), 20% glycerol, 1 μ mol/L β -mercaptoethanol, 15 mmol/L NaF, 1 mmol/L

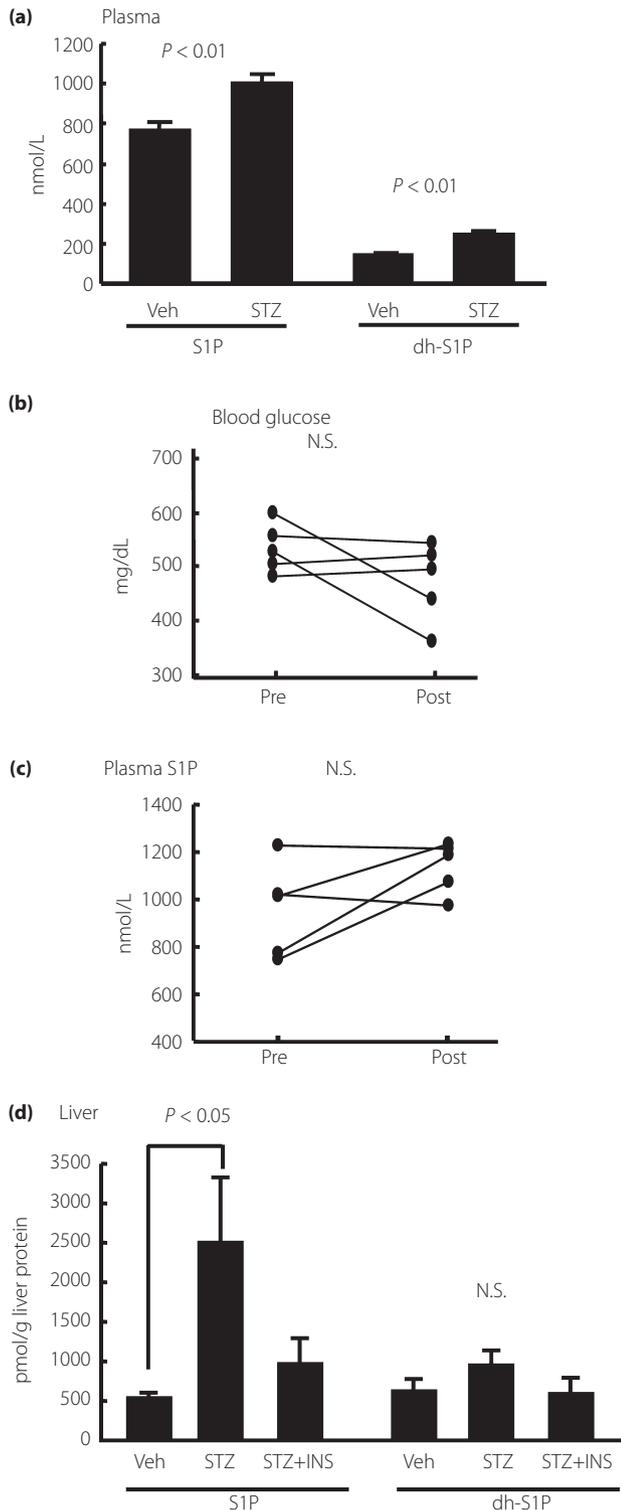


Figure 1 | Modulation of plasma and hepatic sphingosine-1-phosphate (S1P) level in streptozotocin-induced diabetes (STZ) mice. Fourteen-week-old STZ mice or control mice were analyzed after they were subjected to a 6-h fast ($n = 5$ /each group). To investigate the effects of insulin (INS), we administered Humulin R at a dose of 0.75 IU/kg bodyweight; plasma samples were collected before and at 2 h after injection ($n = 5$). (a) Plasma S1P and dihydrosphingosine (dh)S1P levels in STZ mice. (b) Blood glucose and (c) plasma S1P levels before and at 2 h after insulin administration. (d) Liver S1P and dhS1P levels adjusted according to the liver protein level. N.S., not significant; Veh, vehicle.

adenosine triphosphate (Sigma Aldrich Co.) to 300 mg of protein in a final volume of 500 μ L. After incubation at 37°C for 20 min, we terminated the reaction by adding 50 μ L of 1 mol/L HCl and 3 mL of methanol and chloroform (v/v 2:1). Then, the C_{17} -S1P levels were measured as described later.

Measurement of S1P, Dihydrosphingosine-1-Phosphate and C_{17} -S1P

The contents of S1P and dihydrosphingosine-1-phosphate (dhS1P) in the plasma, liver and kidneys were determined using two-step lipid extraction followed by high-performance liquid chromatography separation, as described previously¹⁶. Briefly, samples were sonicated in 3 mL of methanol/chloroform (2:1) with an internal standard for 30 min. After adding 2 mL of chloroform, 2.1 mL of 1 mmol/L KCl, and 100 μ L of 3 N NaOH, the samples were centrifuged and the alkaline upper phase (3.8 mL) was collected into new tubes, to which 4 mL of chloroform and 200 μ L of concentrated HCl were added. The resultant lower chloroform phases (3.5 mL) that were formed under these new acidic conditions were collected and evaporated under nitrogen gas, and resolved in methanol, followed by high-performance liquid chromatography separation using a TSKgel ODS-80TM column (Tosoh, Tokyo, Japan). For the measurement of the S1P contents, we used C_{17} -S1P (Avanti Polar Lipids) as an internal standard, whereas for the C_{17} -S1P contents, we used FTY720(S)-phosphate (Cayman Chemical, Ann Arbor, MI, USA).

Western Blotting

Liver and kidney proteins were extracted using radioimmunoprecipitation assay lysis buffer (25 mmol/L Tris HCL [pH 7.6], 1% NP-40, 0.1% sodium dodecylsulfate, 150 mmol/L NaCl, 0.02% sodium deoxycholate, 1 mmol/L orthovanadate, 1 mmol/L phenylmethyl sulfonyl fluoride and protease inhibitor cocktail [Roche]). Western blotting was carried out with 30 μ g of the liver, kidney or cellular proteins using a standard method. For the plasma analysis, a volume corresponding to 0.5 μ L of plasma was subjected to the western blotting analysis. When western blotting was carried out for the analysis of the proteins in the media, the amount of medium applied was adjusted by the cellular protein level. The following antibodies were used: anti-mouse

ethylenediaminetetraacetic acid, 1 mmol/L Na_3VO_4 , 1 mmol/L phenylmethyl sulfonyl fluoride and protease inhibitor cocktail (Roche, Mannheim, Germany). The reaction was initiated by adding 25 mL of 200 mmol/L C_{17} -sphingosine (C_{17} -Sph; Avanti Polar Lipids, Alabaster, AL, USA) and 25 μ L of 20 mmol/L

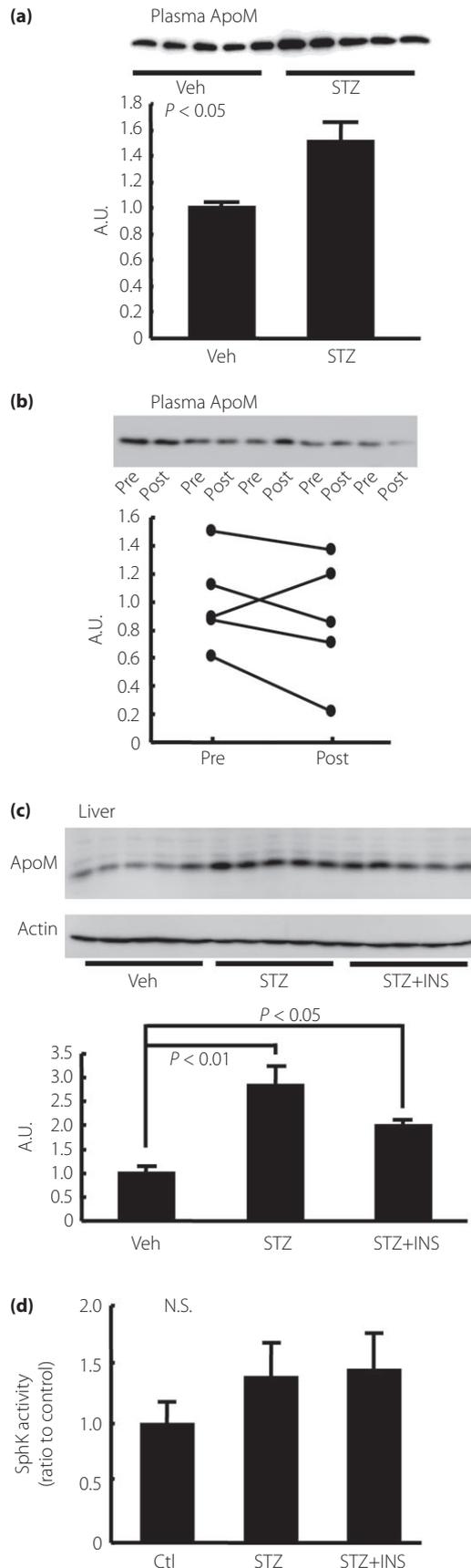


Figure 2 | Modulation of plasma and hepatic apolipoprotein M (ApoM) level in streptozotocin-induced diabetes (STZ) mice. Fourteen-week-old STZ mice or control (Ctl) mice were analyzed after they were subjected to a 6-h fast ($n = 5$ /each group). To investigate the effects of insulin (INS), we administered Humulin R at a dose of 0.75 IU/kg bodyweight; plasma samples were collected before and at 2 h after injection ($n = 5$). (a) Plasma ApoM level and (b) plasma ApoM level before and at 2 h after insulin administration. (c) Liver ApoM level adjusted to the β -actin level. (d) Sphingosine kinase (SphK) activity in the liver. Veh, vehicle; Ctl, control.

ApoM antibody (GenScript Co, Piscataway, NJ, USA), anti-human ApoM serum (developed in a previously reported paper¹²) and anti- β -actin antibody (MBL, Nagoya, Japan).

Statistical Analysis

The results are expressed as the mean \pm SEM. Differences between two independent groups were evaluated using the Student's *t*-test, and differences among more than two independent groups were assessed using a one-way ANOVA, followed by multiple comparison tests. The differences in the means between before and after treatment with reagents within a given group were assessed by paired *t*-test. *P*-values < 0.05 were deemed statistically significant.

RESULTS

Plasma and Liver S1P Levels Increased in STZ Mice

To investigate the modulation of the S1P level in STZ mice, we measured the S1P levels in plasma and liver samples from STZ mice. In agreement with a previous paper⁹, the plasma S1P level was significantly elevated in STZ mice (Figure 1a). In our experiment, the plasma dhS1P concentration was also higher (Figure 1a). Because insulin secretion from β -cells was disturbed in STZ mice, we investigated the effects of insulin on the plasma S1P level. The changes in the blood glucose level before and at 2 h after administration are shown in Figure 1b. With insulin treatment at a dose of 0.75 IU/kg bodyweight, we found that no modulation was observed (Figure 1c). Regarding the modulation of the liver S1P content, the content was remarkably increased in STZ mice and tended to be decreased by insulin treatment (Figure 1d).

Plasma and Liver ApoM Levels Increased in STZ Mice

Next, we investigated whether the ApoM level was modulated in STZ mice. Contrary to a previous report describing alloxan-induced diabetic mice¹³, the plasma ApoM level was higher in the STZ mice (Figure 2a), while the hepatic ApoM level was also elevated (Figure 2c). Although insulin treatment did not restore the increased plasma ApoM level significantly (Figure 2b), the hepatic ApoM content was decreased with insulin treatment (Figure 2c). We also measured the activity of

SphK in the liver, and found that SphK activity was almost unchanged in the STZ mice or by insulin treatment (Figure 2d).

Insulin Treatment at a High Dose Decreased Plasma S1P and ApoM Levels in STZ Mice

As treatment with Humulin R at a dose of 0.75 IU/kg bodyweight did not significantly decrease the blood glucose levels, we next treated STZ mice intraperitoneally with Humulin R at a dose of 2 IU/kg bodyweight, or injected subcutaneously with Humulin N at a dose of 80 IU/kg bodyweight. As shown in Figure 3, although Humulin R at a dose of 2 IU/kg bodyweight

did not decrease the plasma S1P or ApoM levels (Figure 3b,c), Humulin N at a dose of 80 IU/kg bodyweight significantly decreased both the plasma S1P and ApoM levels (Figure 3e,f), maybe because only Humulin N at 80 IU/kg bodyweight decreased blood glucose sufficiently (Figure 3d).

Plasma ApoM Level was Decreased by Insulin Administration in Normal Mice

We have shown, so far, that both S1P and ApoM levels in the plasma and liver were elevated in STZ mice; however, insulin supplementation only restored the elevated S1P and ApoM levels in the plasma when we treated STZ mice with a rather high

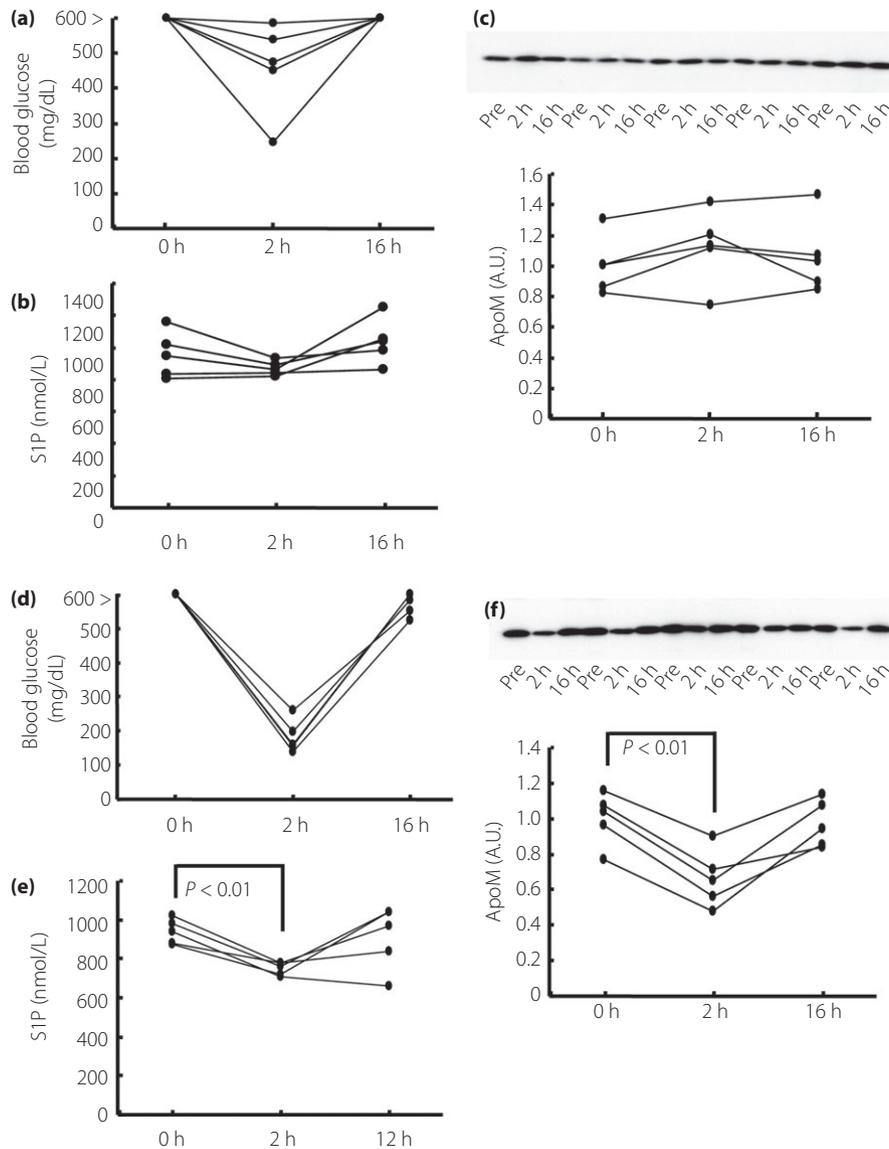


Figure 3 | Treatment with insulin at a high dose decreased plasma sphingosine-1-phosphate (S1P) and apolipoprotein M (ApoM) levels in streptozotocin-induced diabetes (STZ) mice. Twelve-week-old STZ mice were treated with Humulin R (2 IU/kg of bodyweight) or Humulin N (80 IU/kg of bodyweight; $n = 5$ /group). Before and at 2 and 16 h after the administration, the plasma samples were collected and used to measure the S1P and ApoM levels. (a,d) Changes in the blood glucose level, (b,e) changes in the plasma S1P level and (c,f) changes in the plasma ApoM level. A.U., arbitrary unit.

dose of insulin. To reconfirm the effects of insulin on the plasma S1P and ApoM levels, we next investigated whether insulin treatment might modulate the S1P and ApoM levels in mice with normal blood glucose levels. We also investigated the effects of glucose on the S1P and ApoM levels, as the ApoM level is reportedly decreased under high glucose conditions *in vitro*¹⁴. The changes in the blood glucose levels are shown in Figure 4a–c. As shown in Figure 4d–f, the plasma S1P level was reduced significantly by the administration of glucose and the administration of insulin, with the decrease in the insulin-treated group being more apparent. The plasma ApoM level was reduced significantly only in the insulin-treated group (Figure 4g–i). The hepatic ApoM content was also decreased in the insulin-treated group, although the hepatic S1P content was not altered (Figure 5a,b).

Insulin Treatment Did Not Decrease Plasma S1P Levels in ApoM-Overexpressing Mice

To further investigate the role of ApoM in the modulation of S1P by insulin, we used mice overexpressing ApoM by exogenous adenovirus gene transfer (Figure 6a,b). Although insulin treatment with 0.5 IU/kg bodyweight of Humulin R decreased

the blood glucose levels significantly, insulin did not decrease the plasma S1P levels (Figure 6c–e). These results suggest that the modulation by insulin of the plasma S1P levels can be observed under the changes of the plasma ApoM levels within the physiological range.

Incubation with Insulin or in a Low-Glucose Medium Decreased ApoM Levels in HepG2 Cells

We next investigated the effects of insulin, high glucose or low glucose *in vitro* with HepG2 cells, human hepatoma cell line. As shown in Figure 7a,b, insulin decreased both cellular and medium ApoM levels, whereas high glucose did not exert apparent effects on ApoM. As shown in Figure 7c,d, incubation in a low-glucose medium decreased both cellular and medium ApoM levels.

Kidney S1P and ApoM Levels Increased in STZ Mice

ApoM is expressed in the kidney as well as the liver, although plasma ApoM is derived mainly from the liver. Therefore, we next investigated the kidney levels of S1P and ApoM in STZ mice. As shown in Figure 8a, the kidney S1P levels were significantly increased in the STZ mice and tended to be reduced by

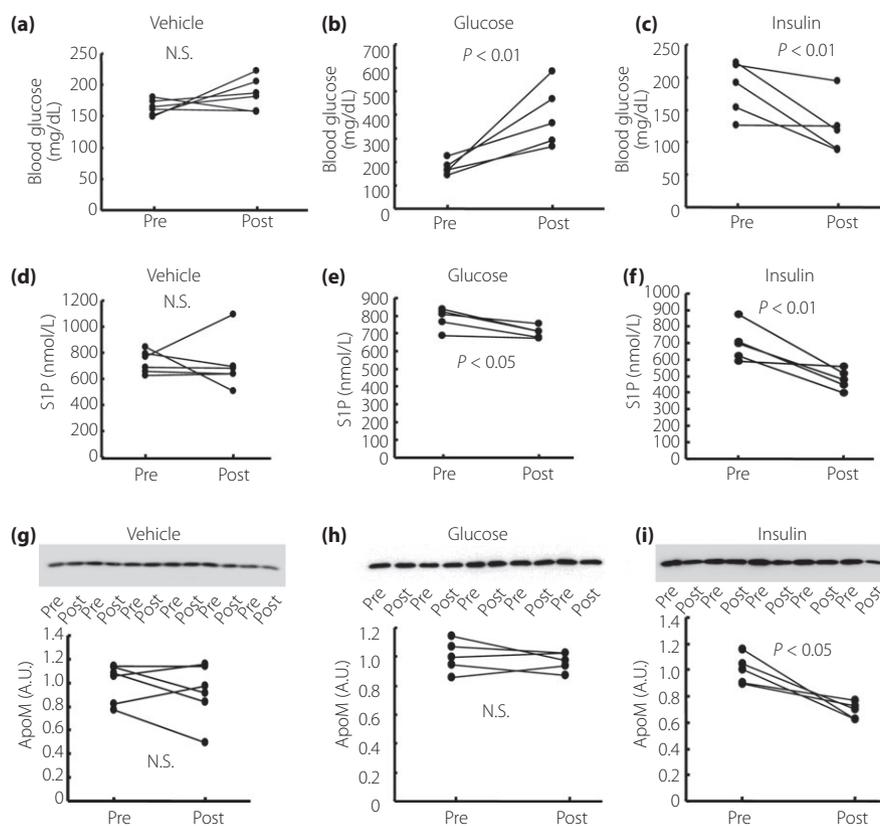


Figure 4 | The effects of insulin and glucose on plasma sphingosine-1-phosphate (S1P) and apolipoprotein M (ApoM) levels in normal mice. Ten-week-old normal mice were treated with Humulin R (0.5 IU/kg of bodyweight), glucose (2 g/kg of bodyweight) or phosphate-buffered saline ($n = 5$ –6/group). Before and at 2 h after the administration, the plasma and liver samples were collected and used to measure the S1P and ApoM levels. (a–c) Changes in the blood glucose level, (d–f) changes in the plasma S1P level and (g–i) changes in the plasma ApoM level. A.U., arbitrary unit; N.S., not significant.

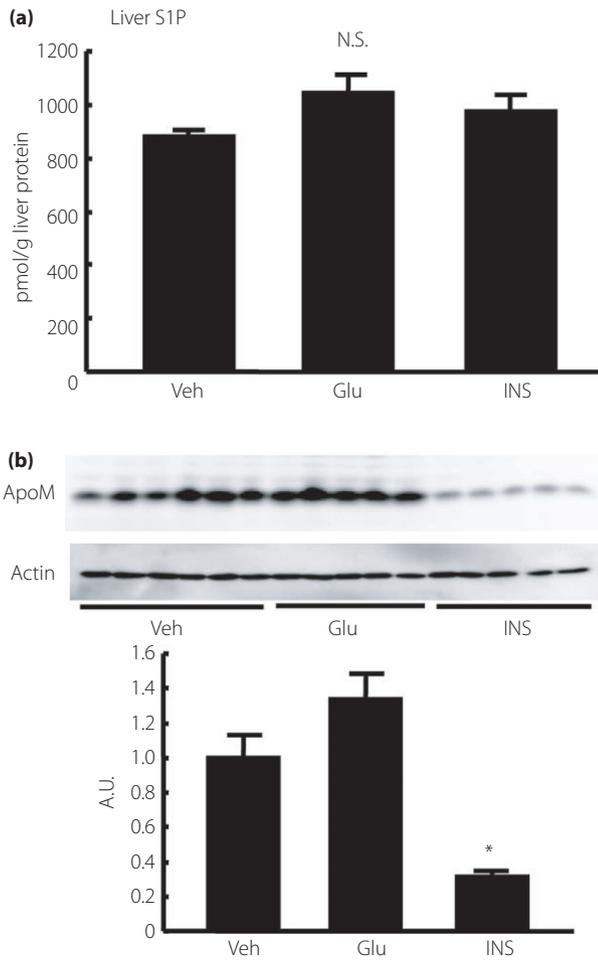


Figure 5 | The effects of insulin (INS) and glucose (GLU) on liver sphingosine-1-phosphate (S1P) and apolipoprotein M (ApoM) levels in normal mice. Ten-week-old normal mice were treated with Humulin R (0.5 IU/kg of bodyweight), glucose (2 g/kg of bodyweight) or phosphate-buffered saline ($n = 5-6$ /group). Before and at 2 h after the administration, the plasma and liver samples were collected and used to measure the S1P and ApoM levels. (a) Liver S1P level adjusted according to the liver protein level, and (b) liver ApoM level adjusted according to the β -actin level. * $P < 0.01$ vs the other groups. A.U., arbitrary unit; N.S., not significant; Veh, vehicle.

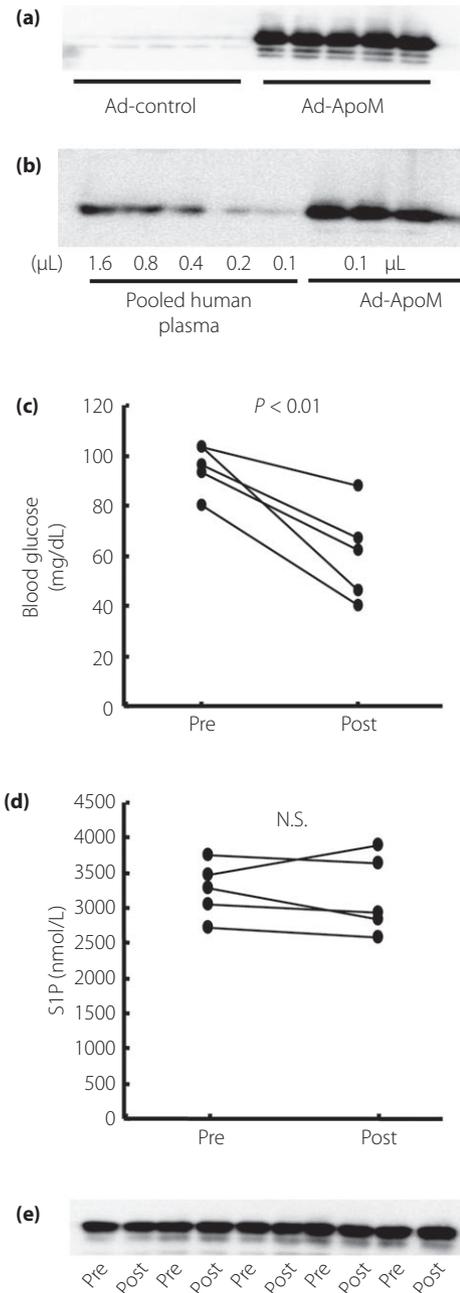
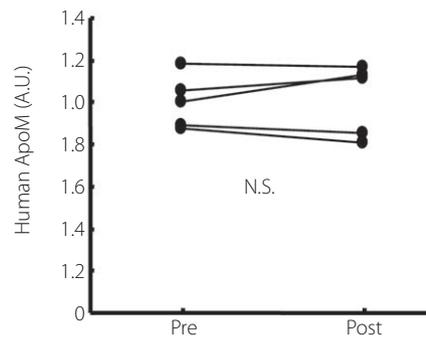


Figure 6 | The effects of insulin on plasma sphingosine-1-phosphate (S1P) and apolipoprotein M (ApoM) levels in ApoM-overexpressing mice. Ten-week-old normal mice were infected with adenovirus coding human ApoM (Ad-ApoM; $n = 5$). At 5 days after the administration, they were treated with Humulin R (0.5 IU/kg of bodyweight). (a) ApoM levels in mice infected with Ad-ApoM and mice infected with control blank adenovirus (Ad-control), (b) the expression level of ApoM in ApoM-overexpressing mice, compared with pooled human plasma. (c) Changes in the blood glucose level, (d) changes in the plasma S1P level and (e) changes in the plasma human ApoM level. A.U., arbitrary unit; N.S., not significant.



insulin treatment. The ApoM content in the kidney was also increased in the STZ mice, and this increment was restored by insulin treatment (Figure 8b). These results showed that a similar modulation of S1P and ApoM in the liver might also exist in the kidney.

DISCUSSION

S1P is a bioactive lipid mediator that is involved in many diseases⁵. Among these diseases, S1P has been shown to be involved in the pathogenesis of diabetes, as S1P has protective properties for β -cell functions. Recently, ApoM, a minor apolipoprotein riding on high-density lipoprotein¹¹, was shown to be a carrier and a modulator of S1P^{10,12}. Clinical studies have also shown that single-nucleotide polymorphisms of ApoM are related to diabetes^{17–19}. Furthermore, the diabetic condition itself might influence S1P and ApoM. In the present study, we investigated the S1P and ApoM levels in the plasma and liver of STZ mice, and found that both the S1P and ApoM levels were elevated in both the plasma and liver. Although the source of increased S1P was unclear, we showed that ApoM retards the degradation of S1P, and that ApoM increases the total S1P mass in the body in a previous study¹², and this result suggests

that the increase in the ApoM level might be deeply involved in the elevation of both the plasma and liver S1P levels in the present study.

Regarding the regulation of ApoM, the involvement of several factors has been proposed: leptin increased ApoM¹³, whereas glucose¹⁴, lipopolysaccharides²⁰ and an liver X receptor agonist²¹ decreased ApoM. In the present study, although the underlying mechanism responsible for the modulation of ApoM remains to be elucidated, from the results that S1P and ApoM increased in STZ mice and that insulin treatment restored the increased levels of S1P and ApoM levels, insulin might have a negative regulatory effect on S1P and ApoM. Although these results are consistent with a report describing the effects in HepG2 cells¹⁴, which was reproduced in the present report (Figure 7a,b), our results conflict with those of a previous report describing alloxan-induced diabetic mice¹³. While β -cell insufficiency is also involved in the pathogenesis of diabetes in alloxan-induced diabetic mice, as well as in STZ mice, there might be two major differences between our mouse model and the previously reported mice. The first difference is that the mice showed a more apparent insulin insufficiency phenotype in the present study; the STZ mice exhibited a

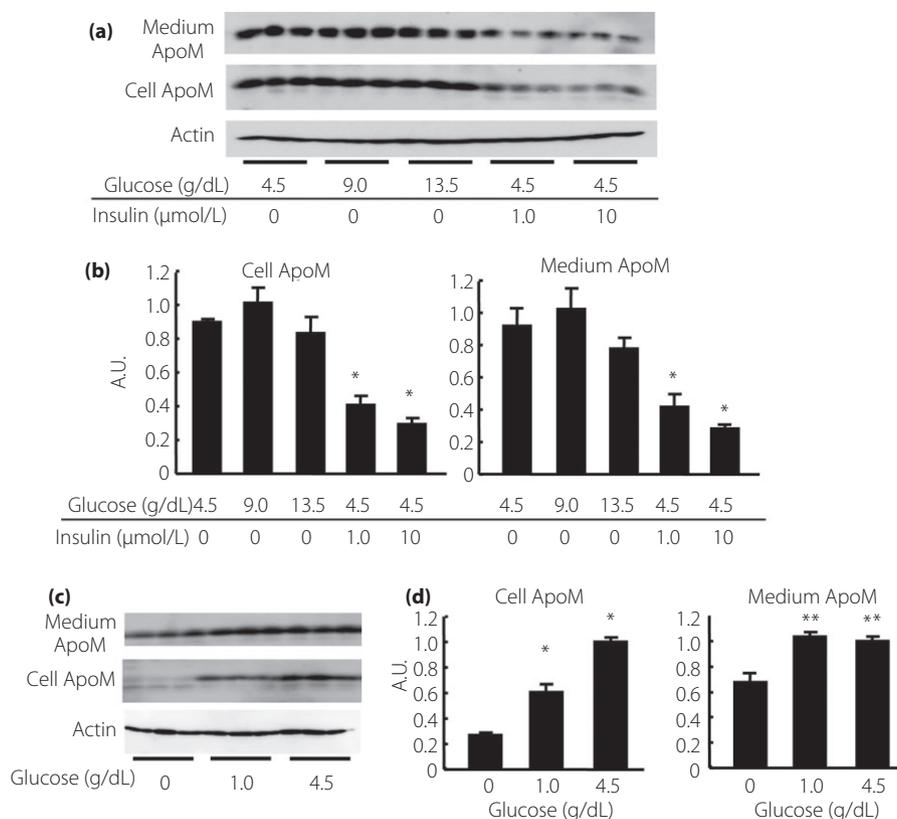


Figure 7 | Modulation of apolipoprotein M (ApoM) levels by insulin and glucose in HepG2 cells. At approximately 70% confluence, HepG2 cells were incubated in fetal bovine serum-free Dulbecco's modified Eagle's medium containing various doses of glucose with or without human insulin solution for 8 h, then the media and cellular components were collected, and medium ApoM and cellular ApoM levels were analyzed. (a,b) The effects of insulin on the ApoM levels in HepG2 cells. $*P < 0.01$ vs HepG2 cells incubated without insulin. (c,d) The effects of the depletion of glucose on the ApoM levels in HepG2 cells. $*P < 0.01$ vs the other groups. $*P < 0.05$ vs HepG2 cells incubated without glucose. A.U., arbitrary unit.

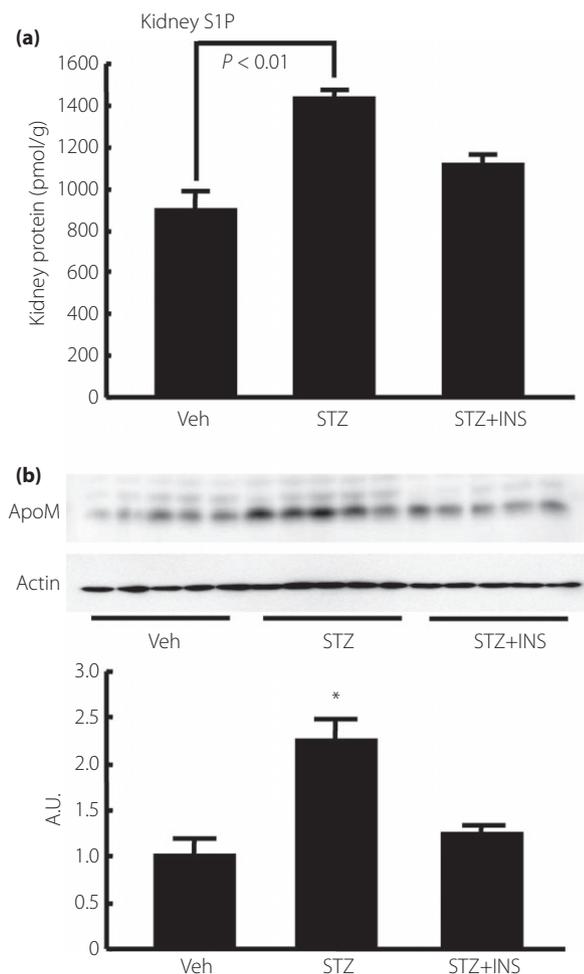


Figure 8 | Modulation of kidney sphingosine-1-phosphate (S1P) and apolipoprotein M (ApoM) contents in streptozotocin-induced diabetes (STZ) mice. Fourteen-week-old STZ mice were analyzed after being subjected to a 6-h fast. To investigate the effects of insulin (INS), we administered Humulin R at a dose of 0.75 U/kg bodyweight; samples were collected at 2 h after the administration ($n = 5/\text{group}$). (a) Kidney S1P level adjusted according to the kidney protein level, and (b) kidney ApoM level adjusted according to the β -actin level. * $P < 0.01$ vs the other groups. A.U., arbitrary unit; Veh, vehicle.

reduced bodyweight, reflecting the loss of fat tissue because of increased lipolysis (Table 1). However, in alloxan-induced diabetic mice, no difference in bodyweight was observed. Therefore, the extent of the insulin insufficiency and the resultant augmentation of lipolysis were more obvious in our STZ mice. The second difference is that the term of exposure to the diabetic conditions was 8 weeks in the present study, but just 3 days in the previous study, which might not have been long enough time to induce an insulin-deficient condition, such as severe lipolysis. Another interesting finding regarding the regulation of ApoM was that the depletion of glucose decreased ApoM protein levels (Figure 7c,d). Although this result apparently conflicts with the previous report, which showed that glucose

decreased the ApoM messenger ribonucleic acid levels in HepG2 cells¹⁴, the present study showed that the ApoM protein levels were decreased when glucose in the media was depleted. Furthermore, close observation of the band of cellular ApoM in Figure 7c showed that the ApoM band in glucose-depleted medium appeared at a lower molecular weight. As human ApoM is highly glycosylated, while murine ApoM is not²², glucose as well as insulin could have important roles in regulating ApoM through increasing the susceptibility of ApoM to glycosylation. Further studies on the effects of other diabetic factors, such as free fatty acids, on ApoM and S1P are required.

The negative regulation of ApoM and S1P by insulin in the present study suggests that the liver might have some roles in maintaining the homeostasis of glucose metabolism. Considering the protective properties of S1P on β -cells, the results from the present study suggested that when β -cell functions were disturbed and insulin levels in the circulation were lowered, ApoM levels in the liver increased, and this compensatory modulation of ApoM results in an increased S1P level, leading to the protection of β -cell functions.

Another interesting result of the present study was that the kidney S1P and ApoM levels were also elevated in STZ mice, and insulin treatment restored this elevation. In the kidney, ApoM is expressed in tubular cells²³, and ApoM is excreted into the urine and reabsorbed at the proximal tubule with megalin as receptors²⁴. Although the mechanism by which ApoM in the kidney contributes to the renal S1P content is unclear, the changes in the renal ApoM and S1P contents observed in STZ mice might be one of the compensatory pathways to protect the kidney against injuries caused by diabetes, as S1P has been shown to protect the kidney in recent studies²⁵.

In conclusion, both ApoM and S1P in the plasma, liver and kidney were increased in STZ mice, possibly because of insulin deficiency. ApoM, which is produced in the liver, might have an important role in maintaining the homeostasis of β -cell functions by increasing the S1P level.

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