# Rosiglitazone attenuates casein-induced hepatic endoplasmic reticulum stress in Sprague-Dawley rats: a novel model of endoplasmic reticulum stress

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**Abstract.** The proteins found in cow milk have been reported to cause systemic inflammation. Endoplasmic reticulum (ER) stress is known to be involved in the development of several metabolic disorders including insulin resistance and non-alcoholic fatty liver disease. However, the effect of thiazolidinediones (TZDs) on ER stress is still controversial. This is why we want to investigate in this study whether casein, which is the major protein in cow's milk, induces ER stress in the liver and whether rosiglitazone can attenuate these changes. Nine-week-old male Sprague-Dawley (SD) rats were separated into three groups: (1) vehicle treated; (2) daily subcutaneous injections of 1 mL 10% casein; (3) daily subcutaneous injection of 1mL 10% casein and rosiglitazone 4 mg/[kg d]. After 6 weeks, body weight, food intake, glucose and lipid parameters, and serum AST/ALT levels were measured after an overnight fast. Real time RT-PCR and immunohistochemical staining for various ER stress markers were performed, and a TUNEL analysis was also performed. After 6 weeks, casein injection induced weight reduction, systemic inflammation, and hepatic dysfunction in SD rats. Casein injection increased both the gene and protein expression of ER stress markers in the liver and also caused hepatocyte apoptosis. Rosiglitazone treatment attenuated casein-induced systemic inflammation, ER stress, deteriorated liver function, and increased apoptosis. In conclusion, our results may provide further insight into the effects of casein on chronic inflammatory diseases, and to have a better understanding of the mechanism of the anti-inflammatory properties of rosiglitazone regardless of its hypoglycemic effect.

*Key words*: Casein, Hepatic endoplasmic reticulum stress, Rosiglitazone

**THE ENDOPLASMIC RETICULUM** (ER) is the site of synthesis, folding and maturation of secreted and transmembrane proteins [1]. ER stress is defined as an imbalance between the protein folding capacity of the ER and the client protein load, resulting in the accumulation of misfolded proteins. Several recent research studies have proposed that ER stress is a cause of chronic inflammatory disorders and chronic metabolic diseases, including type 2 diabetes, obesity, and non-alcoholic fatty liver disease (NAFLD) [2, 3]. The proteins in cow milk have been reported to cause systemic inflammation that is triggered by the immune response system.

According to recent reports, early consumption of cow's milk may be a risk factor for the development of autoimmune disease such as multiple sclerosis, mild rheumatoid arthritis in rabbits, and type 1 diabetes [4, 5]. However, the physiological mechanism of the inflammatory effect that is induced by the proteins found in cow's milk is not well-defined. Casein is the most abundant protein in cow milk, and many research studies have focused on this protein and its inflammatory effect. The effects of mitogenesis [6], immunoglobulin production [7], and augmentation of phagocytosis [8] have especially been studied well. However, there has been limited study about casein and induction of ER stress.

Thiazolidinediones (TZDs), known anti-diabetic agents, are well established insulin sensitizing agents that act, at least in part, as agonists of the peroxisome proliferator-activated receptor (PPAR) gamma. These drugs not only have a peripheral insulin sensitizing

effect in adipose tissue, but also anti-inflammatory and anti-apoptotic effects [9]. Several reports show the effects of TZDs on ER stress and chronic inflammation. Loffler *et al.* [10] found significant down-regulation of ER stress genes in the livers of db/db mice treated with rosiglitazone. Additionally, Han *et al.* [11] have demonstrated that reduced ER stress in the livers and adipose tissues in the same rodent model treated with the dual PPARα/γ agonist macelignan. In contrast, pioglitazone failed to protect either the HepG2 hepatocyte or adipocyte cell lines from ER stress induced by a variety of mechanisms [12]. Thus, the effect of TZDs on ER stress is still controversial.

So in this study, we want to investigate whether induction of ER stress is one of the underlying mechanisms of chronic inflammation caused by casein. Furthermore, we tried to confirm the positive effect of rosiglitazone in the situation of increased ER stress.

## Materials and Methods

### Animals, diet, and treatment

Nine-week-old male Sprague-Dawley (SD) rats (Central Laboratory Animal, Seoul, Korea) were maintained at ambient temperature (22°C ± 1°C) on 12-hour light-dark cycles with free access to water and food. The SD rats were fed a chow diet and were separated into three groups: (1) vehicle treated (n=10); (2) daily subcutaneous injections of 1 mL 10% casein (n=10); (3) daily subcutaneous injection of 1 mL 10% casein and rosiglitazone (Avandia, GlaxoSmithKlin, USA; 4 mg/[kg d]). Rosiglitazone was dissolved in 1 mL of drinking water and was administered orally with a 10-mL syringe for 6 weeks starting when the rats were 9 weeks of age. The chow diet contained 11.9% of energy from fat and was purchased from LabDiet® (USA). The rats were sacrificed at 15 weeks of age. The animals were euthanized at the end of a dark cycle after they had fasted overnight in order to obtain optimal tissue sampling. Blood was collected by cardiac puncture. The livers were isolated, immediately freeze-clamped in liquid nitrogen, and stored at −80 °C until the time of analysis. All of the procedures were approved by the Institutional Animal Care and Use Committee at the Yonsei University College of Medicine.

### Biochemical analysis

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (T-CHO) and triglyceride (TG) levels were quantified in the serum (Biosource Invitrogen, Camarillo, CA, USA). Serum insulin levels were measured using a commercial kit (ALPCO Diagnostics, Windham, NH, USA). Serum concentrations of various cytokines, including TNF-a (R&D Systems, Minneapolis, MN), IL-1β (R&D Systems, Minneapolis, MN), G-CSF (USCN Life Science Inc., Wuhan, China), and IL-6 (ID Labs, London, Ontario, Canada), were measured using commercially available ELISA tests according to the manufacturer's instruction.

### Cytochrome P450 activity analysis

Hepatic cytochrome P450 activity analysis was performed using a commercially available kit (Arbor Assays, Ann Arbor, MI).

### RNA preparation and RT-PCR

RNA was isolated from the rat liver tissue and the hepatocyte-derived cells using the Trizol reagent (Invitrogen), and the total RNA was quantified using the nanodrop method (ND-1000; DM Science, Seoul, Korea). Following RNA extraction, 4 μL RNA was treated with 1 unit DNase I in order to remove all contaminating genomic DNA. DNase-treated RNA was subsequently used for complementary DNA (cDNA) synthesis using the MMLV reverse transcriptase method (Promega, Madison, WI): 1 μL oligo dT primer was added to 4 μL RNA with 5× MMLV reaction buffer, 2.5 mmol/L each dNTP, 1 unit RNasin ribonuclease inhibitor, and MMLV reverse transcriptase (200 units). Complementary DNA was stored at -20°C.

Real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis was performed with an ABI 7500 instrument and its associated software (Applied Biosystems, Foster City, CA, USA). All of the Taqman assays were performed with inventoried primers and probes (Applied Biosystems): β-actin (Hs99999903_m1, R700667669_m1), ATF6 (Rn01490844_m1), inositol requiring enzyme-1α (IRE-1α) (Rn01427574_m1), XBP-1 (Rn01443523_m1), C/EBP homologous protein (CHOP) (Rn00492098_g1), eIF-2α (Rn01494813_m1), and protein kinase RNA-like ER kinase (PERK) (Rn00571015_m1). PCR reactions were performed in triplicate in a final volume of 20 μL according to the manufacturer's protocol. For each assay, a standard curve was obtained by analyzing a dilution series of pooled cDNA samples for the relevant gene. Data were analyzed with Sequence Detector 1.7 software (Applied Biosystems). β-actin was used as

the internal standard to control for variability since the results were expressed as a ratio of the gene expression relative to that of β-actin.

### Immunoblot analysis

Aliquots of the cell lysates (Thermo Scientific, Waltham, MA) and the tissue homogenates were denatured under reducing conditions (1.75% SDS, 15 mM 2-mercaptoethanol; 5 min at 100°C), and were then used for the SDS-PAGE and immunoblot analyses. Total protein levels were determined using the Bradford assay (Sigma-Aldrich, St. Louis, MO). To detect TNF-α and IL-1β levels in liver tissue, nitrocellulose membranes were first incubated with anti-TNF-α antibody (rabbit monoclonal IgG, Calbiochem, Germany) (1:250, overnight at 4°C) and anti-IL-1β antibody (rabbit monoclonal IgG, Millipore, USA) (1:250, overnight at 4°C) and were then incubated with goat anti-rabbit IgG conjugated to horseradish peroxidase (1:5,000, Santa Cruz Biotechnology, Santa Cruz, USA) for 1 hour. Immunolabeling was detected with the ECL Western Blotting Analysis System (Thermo Scientific, Waltham, MA). β-actin immunoreactivity served as the loading control and was detected when the monoclonal anti-mouse and the goat anti-mouse IgG secondary antibody were conjugated to the horseradish peroxidase (1:5,000, Sigma-Aldrich, St. Louis, MO).

### Immunohistochemical staining analysis

The livers from the rats tested in this study were fixed in 10% formaldehyde and embedded in paraffin. The paraffin sections were cut and deparaffinized using xylene and ethanol. After inactivation of endogenous peroxidase with 3% $H_2O_2$ in methanol for 15 min at room temperature, samples were incubated overnight with anti-IRE-1α (ABNOVA, Taipei City, Taiwan), PERK, GRP78, and CHOP (Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C. The next day, the samples were washed with PBS and were then incubated with the probe labeled with anti-rabbit IgG antibodies and peroxidase for 30 min at room temperature. Sections were counterstained with hematoxylin prior to being examined under a light microscope.

### TUNEL assay

Pretreatment with proteinase K (20 μg/mL, Roche Molecular Biochemicals, Germany) was performed for 15 min, followed by four two-minute washes with distilled water. Endogenous peroxidases were inac-

tivated by incubating the sections in 2% $H_2O_2$ for 5 min at room temperature and then washed in distilled water. Sections were then immersed for 10 min at room temperature in TdT buffer (30 mm Tris-HCl, pH 7±2, 140mm sodium cacodylate, 1mM cobalt chloride), incubated for another hour at 37°C in TdT (50 U, 50 mL; Boehringer Mannheim) and biotinylated dUTP (50 mM, 50 mL) in TdT buffer in a humid atmosphere. The reaction was stopped by transferring the sections to TB buffer (300 mM sodium chloride, 30 M sodium citrate) for 15 min at room temperature. The sections were subsequently rinsed in distilled water and blocked in 2T bovine serum albumin in PBS for 10 min at room temperature. After rinsing in distilled water and immersion in PBS for 5 min, the sections were covered with extravidin-horseradish peroxidase (Sigma) diluted 1:20 in PBS for 30 min at room temperature. These sections were then rinsed twice in PBS (5 min each) and stained for no more than 10 min using diaminobenzidine (DAB) as the chromogen.

### Statistical analysis

All statistical analyses were performed with SPSS software (version 18.0; SPSS, Chicago, IL). Values were expressed as the mean ± SD. Either Student's t test or one-way ANOVA was performed in conjunction with protected *post hoc* tests (Tukey's honestly significant difference) in order to make statistical comparisons between the groups in this study. Data with a *P* value < 0.05 were considered significant.
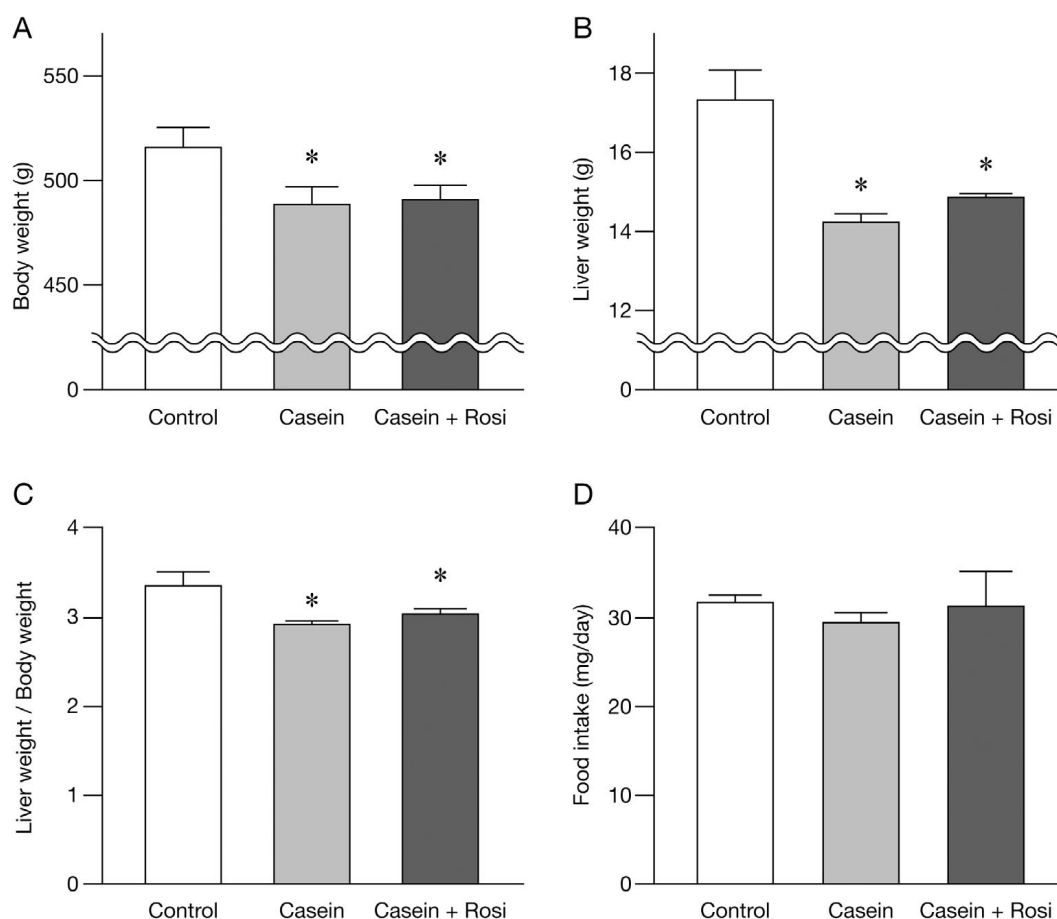
## Results

### Casein injection decreases body weight without reduction in food intake and has no effect on glucose metabolism and lipid parameters.

After 6 weeks of casein injection, the body weight decreased in the casein-injection group compared to that of the control group, and the liver weight decreased as well (515 ± 19 g *vs.* 487 ± 17 g, 17.3 ± 1.3 g *vs.* 14.2 ± 0.4, p = 0.019, p = 0.001, respectively). The food intake was not different among the three groups (31.7 ± 1.4 g/day *vs.* 29.4 ± 1.7 g/day *vs.* 31.1 ± 7.1 g/day, p = 0.594). The casein injection did not affect the fasting plasma glucose, fasting serum insulin, total cholesterol or HDL cholesterol level (Table 1). The body weight, liver weight, glucose and lipid parameters were comparable in both casein-injection group and casein-injection with rosiglitazone treated group (Fig. 1).

**Table 1** Metabolic parameters of Sprague-Dawley rats after 6 weeks of treatment

|  | Control (n = 10) | Casein (n = 10) | Casein + Rosi (n = 10) |
|---|---|---|---|
| Body weight (g) | 515 ± 19 | 487 ± 17* | 489 ± 19* |
| Food intake (mg/day) | 31.7 ± 1.4 | 29.4 ± 1.7 | 31.1 ± 7.1 |
| Liver weight (g) | 17.3 ± 1.3 | 14.2 ± 0.4* | 14.8 ± 0.1* |
| Liver weight / Body weight (%) | 3.36 ± 0.24 | 2.92 ± 0.05* | 3.02 ± 0.10* |
| Fasting plasma glucose (mg/dL) | 120 ± 13 | 121 ± 7 | 117 ± 16 |
| Fasting insulin (μIU/mL) | 0.13 ± 0.05 | 0.09 ± 0.02 | 0.08 ± 0.01 |
| Total cholesterol (ng/mL) | 0.10 ± 0.01 | 0.11 ± 0.01 | 0.10 ± 0.01 |

Data are presented as mean ± SD. *$P < 0.05$ *vs.* control, [†]$P < 0.05$ *vs.* Casein. Control (1 mL of saline injection, n = 10), Casein (1 mL of 10% casein injection, n = 10), Casein + Rosi (1 mL of 10% casein injection and 4 mg/kg of rosiglitazone treatment, n = 10).



**Fig. 1** Body weight and liver weight of Sprague-Dawley rats after 6 weeks of treatment
(A) Body weight, (B) liver weight, (C) liver weight / body weight ratio, (D) food intake after casein injection in Sprague-Dawley rats. *$P < 0.05$ *vs.* control. [†]$P < 0.05$ *vs.* Casein. Results are presented as mean ± SD. Control (1 mL of saline injection, n = 10), Casein (1 mL of 10% casein injection, n = 10), Casein + Rosi (1 mL of 10% casein injection and 4 mg/kg of rosiglitazone treatment, n = 10).
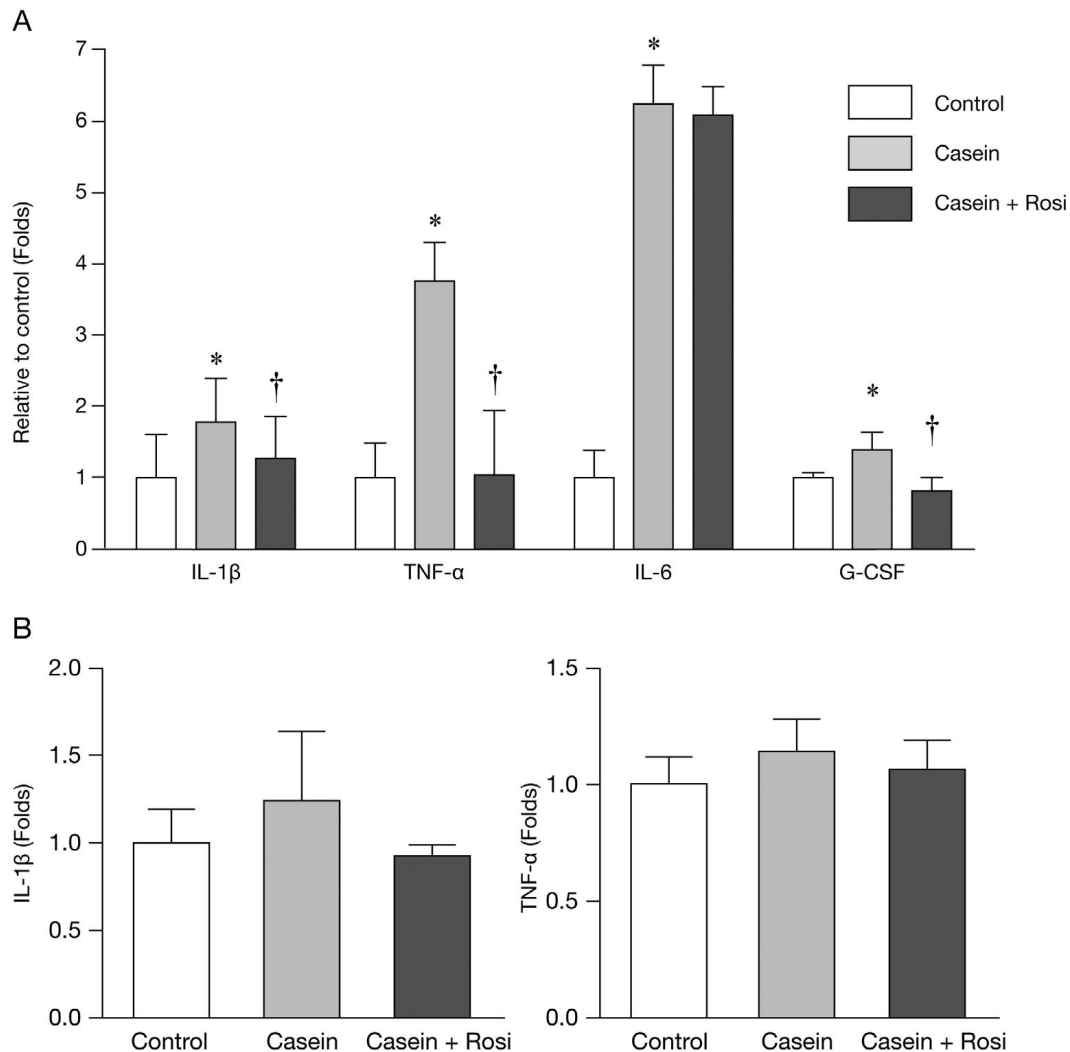
*Casein injection induces systemic inflammation, and rosiglitazone treatment ameliorates the inflammatory stress in SD rats*

Serum concentrations of several cytokines were measured to detect the systemic inflammation after 6 weeks of casein treatment. Serum IL-1β, TNF-α, IL-6, and G-CSF levels increased in the casein injection group (Fig. 2A). This shows that casein injection induced systemic inflammation in SD rats, consistent with the findings of previous reports. We also measured the expression of IL-1β and TNF-α in the liver tissues of these SD rats. The protein levels of IL-1β and TNF-α showed

trend of increase in casein-injection group and trend of decrease when rosiglitazone treated. However, there was no statistical significance (Fig. 2B).

*Casein injection worsens hepatic function in SD rats, and rosiglitazone treatment prevents the deterioration of liver function*

To examine whether casein injection affects the hepatic function in SD rats, we measured the serum levels of AST and ALT and quantified the activity of cytochrome P450 enzymes in the livers of these SD rats. Both AST and ALT levels increased by more than



**Fig. 2** Systemic and local inflammation after casein injection in Sprague-Dawley rats
(A) Serum concentration of IL-1β, TNF-α, IL-6 and G-CSF. *$P < 0.05$ *vs.* control, [†]$P < 0.05$ *vs.* Casein. (B) Protein expression of IL-1β and TNF-α in liver tissue. Results are presented as mean ± SD. Control (1 mL of saline injection, n = 10), Casein (1 mL of 10% casein injection, n = 10), Casein + Rosi (1 mL of 10% casein injection and 4 mg/kg of rosiglitazone treatment, n = 10).
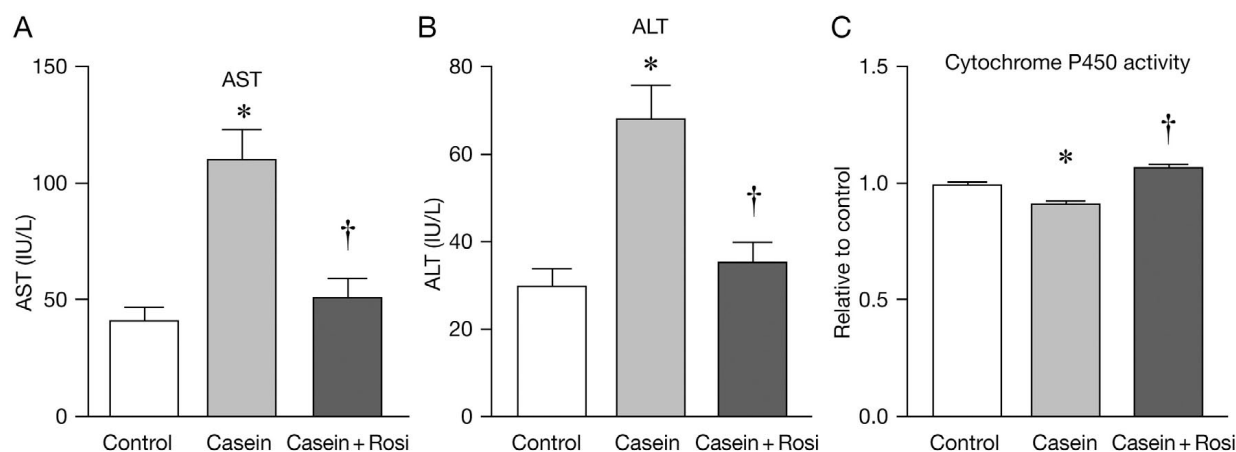
**Fig. 3** Effects of casein injection on hepatic function in Sprague-Dawley rats
(A) Serum AST and (B) ALT levels and (C) cytochrome P450 activity in the liver were measured after 6 weeks of treatment. *$P < 0.05$ *vs.* control, †$P < 0.05$ *vs.* Casein. Results are presented as mean ± SD. Control (1 mL of saline injection, n = 10), Casein (1 mL of 10% casein injection, n = 10), Casein + Rosi (1 mL of 10% casein injection and 4 mg/kg of rosiglitazone treatment, n = 10).

double in casein injection group compared to the control group (40.1 ± 16.7 *vs.* 109.4 ± 41.1 IU/L, 29.6 ± 9.2 *vs.* 68.0 ± 19.7 IU/L, $p < 0.001$, $p < 0.001$, respectively) (Fig. 3A, 3B). However, rosiglitazone treatment prevented the elevation of these liver enzymes (109.4 ± 41.1 *vs.* 49.7 ± 22.7 IU/L, 68.0 ± 19.7 *vs.* 35.0 ± 11.4 IU/L, $p = 0.001$, $p = 0.002$, respectively). The activity of cytochrome P450 was decreased in the casein injection group and was recovered by the rosiglitazone treatment (1.00 ± 0.04 *vs.* 0.89 ± 0.04 *vs.* 1.02 ± 0.08, $p < 0.001$) (Fig. 3C). From these results, we confirmed that chronic casein injection induced hepatic dysfunction and rosiglitazone could prevent that.

***Casein injection induces hepatic ER stress, and rosiglitazone treatment reduces ER stress in the liver***

To investigate the changes of ER stress in the liver after casein-injection, we measured several key transcriptional markers of ER stress: ATF6, IRE-1α, XBP1, CHOP, eIF-2α and PERK (Fig. 4A). The levels of the ER stress gene transcripts were all increased in the casein-injection group. The gene expression of XBP1, CHOP, eIF-2α and PERK decreased in the rosiglitazone-treated group compared to that of the casein-injection group. Regarding ATF6 and IRE-1α levels, the mRNA expression decreased; however, the difference did not reach statistical significance. We also performed an immunohistochemical staining analysis with several ER stress markers, which were GRP78, PERK, IRE-1α and CHOP. These various ER stress markers increased

in the casein-injection group compared to the control group and decreased in the rosiglitazone-treated group, which is consistent with the results of the gene expression (Fig. 4B).

***Casein causes apoptosis in the liver, and rosiglitazone treatment attenuates the casein-induced apoptosis***

Since chronic or severe ER stress can initiate apoptosis, we studied whether casein injection caused apoptosis in the liver. TUNEL staining indicated a large percentage of apoptotic cells in rats injected with casein compared to the control rats. We were also able to determine that the rosiglitazone treatment markedly decreased the apoptotic cell death in the liver (Fig. 5).

## Discussion

In the present study, we demonstrated that casein injection for 6 weeks induced ER stress in the livers of rodents, and that rosiglitazone, an anti-diabetic agent, attenuated the casein-induced ER stress. Our findings include: (1) casein injection can induce weight reduction and systemic inflammation in SD rats; (2) casein injection induces hepatic dysfunction; (3) casein injection increases ER stress in the liver; (4) casein injection increases hepatocyte apoptosis; and (5) rosiglitazone treatment attenuates casein-induced systemic inflammation, ER stress, deteriorated liver function, and increased apoptosis.

The effect of milk and milk proteins on human health
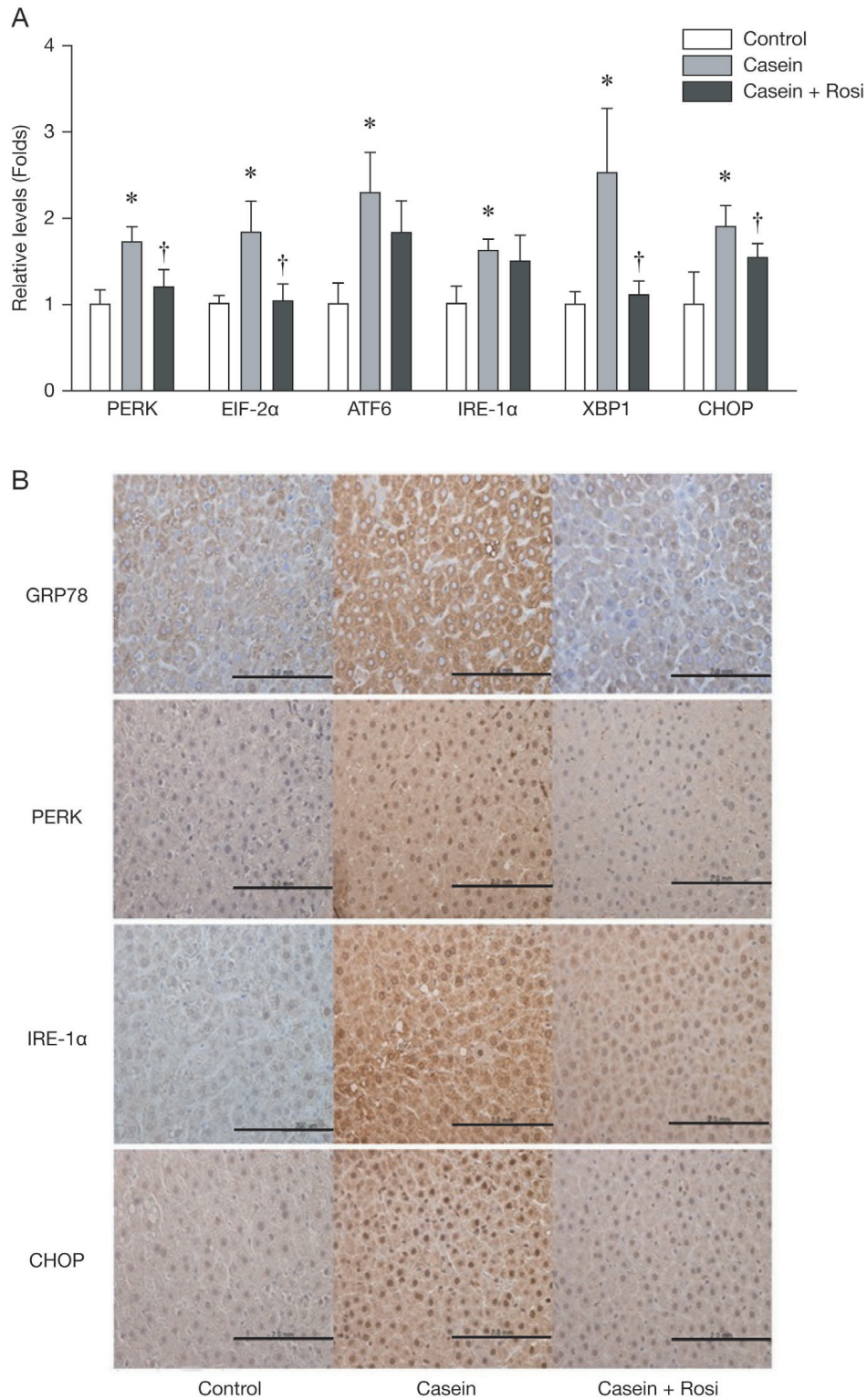
**Fig. 4** Effects of casein injection and/or rosiglitazone treatment on hepatic ER stress markers in Sprague-Dawley rats
(A) qRT-PCR analysis of ATF6, IRE-1a, XBP1, CHOP, EIF-2a, and PERK mRNA expression. *$P < 0.05$ *vs.* control, [†]$P < 0.05$ *vs.* Casein. (B) Immunohistochemical staining of the liver with the following ER stress markers: GRP78, PERK, IRE-1α, and CHOP. Control (1 mL of saline injection, n = 10), Casein (1 mL of 10% casein injection, n = 10), Casein + Rosi (1 mL of 10% casein injection and 4 mg/kg of rosiglitazone treatment, n = 10). Bars indicate 2.0 mm.
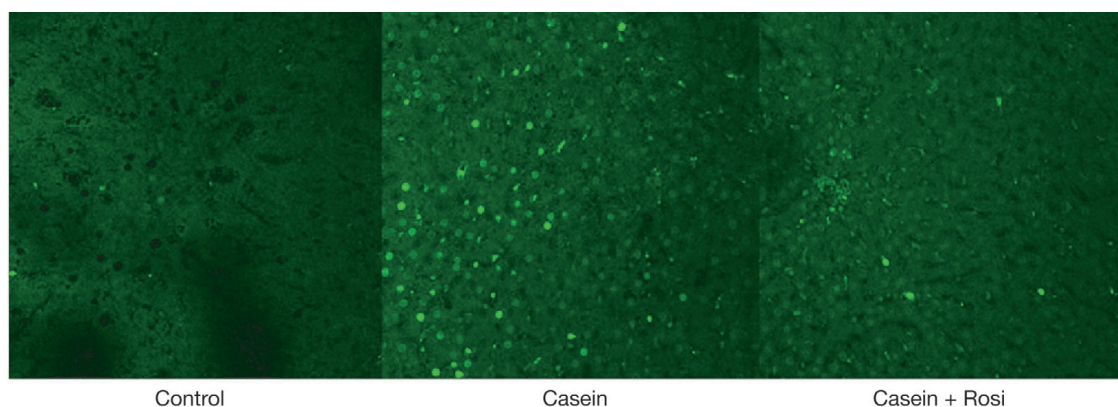
**Fig. 5** Effects of casein injection on hepatocyte apoptosis in Sprague-Dawley rats.
Representative images from the TUNEL staining of liver sections. Control (1 mL of saline injection), Casein (1 mL of 10% casein injection), Casein + Rosi (1 mL of 10% casein injection and 4 mg/kg of rosiglitazone treatment). (×200)

remains controversial [13, 14]. The casein protein represents 80% of the total protein in cow's milk, and 35% of the casein is β casein. There is an extensive body of evidence that specifically links A1 β casein to a range of illnesses, such as type 1 diabetes mellitus, ischemic heart disease, and several autoimmune diseases [15-18]. The mechanism of casein-induced inflammation is not clear; however, increased vascular permeability, leukocyte infiltration, and release of various mediators, such as IL-1 and G-CSF, are thought to play important roles in its pathogenesis [5, 19]. However, there is a lack of research on ER stress and casein intake or exposure to casein. ER stress has been implicated in chronic diseases and conditions involving inflammation, which includes diabetes, obesity, neurodegenerative diseases, and inflammatory bowel diseases [20-22]. ER is an initial checkpoint for the folding and modification of proteins that reside within the secretory pathway, as only properly folded and modified proteins can exit in the ER [23]. The unfolded protein response (UPR) signaling pathway plays a major role in this regulation by sensing the ER lumen environment and transmitting this information to the nucleus in order to up-regulate the transcription of genes that increase both the ER protein folding and its secretory capacities [24]. In mammalian cells, the UPR response is induced by three initiator/sensor molecules on the ER membrane, which are IRE-1, PERK, and ATF6. Each sensor undergoes activation in response to elevated levels of unfolded proteins. Recently, Ma *et al.* [25] have suggested the use of casein-injected apolipoprotein E knockout mice as a novel animal model for the early stages of NAFLD.

The mice were injected subcutaneously with 0.5 mL 10% casein and were fed a Western diet containing 21% fat for 8 weeks. There was a significant increase of serum inflammatory markers like SAA and TNF-α compared with controls, and the hepatic mRNA and protein expression of TNF-α and MCP-1 in the livers of casein-injected mice were significantly increased when stress was successfully induced. Neither ALT nor AST were elevated, and there was no obvious fibrosis in either the control or casein injection group. However, this study did not measure the changes in expression of ER stress marker genes or proteins. In our study, after 6 weeks of daily casein injections, the gene transcription of all of the ER stress markers had increased, and we confirmed the protein expression of these markers in the liver using immunohistochemical staining analysis. We suggest that casein injection could be used as one method to induce ER stress in an animal model.

It is well known that ER stress is involved in the development of insulin resistance and type 2 diabetes mellitus, and it is also known that TZDs are anti-diabetic agents that act as a potent insulin sensitizer. This is why there have been many efforts to investigate the effect of TZDs on ER stress. Pirot *et al.* [26] have demonstrated that a differential response of pancreatic β-cells exists to diverse ER stress inducers, such as various cytokines and palmitates, which leads to a differential regulation of the gene expression of CHOP, an ER stress marker. Hepatocytes may be a more important source of ER stress, especially in cases of insulin resistance [27], as there are more studies on the liver than another organs. Han *et al.* showed reduced liver and

white adipose ER stress in diabetic *db/db* mice treated with the dual PPARα/γ agonist macelignan. This agent also suppressed thapsigargin-induced ER stress at the cellular level in mouse hepatocytes and adipocytes [11]. Loffler *et al.* have also found significant down-regulation of ER stress genes in the livers from diabetic *db/db* mice treated with rosiglitazone [10]. Yoshiuchi *et al.* have reported that pioglitazone treatment suppressed ER stress in the liver by using ER-stress-activated indicator transgenic mice [28]. This is why they had suggested that the suppression of ER stress might explain, at least in part, the pharmacological effects of pioglitazone in reducing insulin resistance. In contrast to these reports, Das *et al.* have suggested that improved insulin sensitivity with pioglitazone is not mediated by a reduction in ER stress [12]. They were also able to confirm the results using clinical data in human hepatocytes by using the HepG2 cell line. The discrepancy in these findings and our results may be due to the differences in the ER stressor, the inherent characteristics of different drugs, or the different doses of drugs that were used.

There are several limitations of our study. Firstly, we could not evaluate the status of ER stress in other peripheral tissues such as skeletal muscle or adipose tissue, and thus we were unable to tell whether the casein injection had an effect on peripheral energy metabolism. The casein injection resulted in prominent systemic inflammation as shown in increased serum levels of inflammatory cytokines, however, it did not increased protein expression of IL-1β and TNF-α in liver tissue significantly. So, chronic inflammation or changes of energy metabolism in another peripheral tissue might involve the casein-induced systemic inflammation. Secondly, the underlying molecular mechanism of how casein induces ER stress or how rosiglitazone attenuates this change was not clearly defined in our study. It may be possible that increased levels of serum adiponectin influenced the reduction of ER stress in the liver, [28]. Lastly, we did not quantify the degree of casein-induced ER stress and hepatocyte apoptosis. We only observed the change of protein expressin of ER stress marker using immunohistochemical stain only.

## Conclusion

In conclusion, we found that casein injection induced ER stress in the liver, worsened liver function, and caused cellular apoptosis. We also determined that rosiglitazone treatment prevented the casein-induced ER stress and the concomitant changes. Our results may provide further insight into the effects of casein on chronic inflammatory diseases and a better understanding of the underlying mechanism of the anti-inflammatory action of rosiglitazone regardless of its hypoglycemic effect.

## Acknowledgement

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