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Original Article

# *Trans*-Resveratrol Enhances the Anticoagulant Activity of Warfarin in a Mouse Model

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***Aim*: Resveratrol is a popular ingredient in dietary supplements. Some patients concomitantly use dietary supplements and medicines in Japan. In the present study, we determined whether *trans*-resveratrol and melinjo (*Gnetum gnemon* L.) seed extract (MSE), which contains resveratrol dimers, interacted with drugs using a mouse model.**

***Methods*: Male C57BL/6J mice were fed experimental diets containing 0.005%, 0.05%, or 0.5% (w/w) *trans*-resveratrol or MSE for 1 or 12 weeks. The expression of liver cytochrome P-450 (CYP) mRNA and activity of liver microsomal CYP were measured. To determine the influence of resveratrol or MSE on drug efficacy, the anticoagulant activity of warfarin was examined in mice that were fed diets containing *trans*-resveratrol or MSE for 12 weeks.**

***Results*: When the mice were fed experimental diets for 1 week, none of the doses of *trans*-resveratrol and MSE affected body weight, liver weight, or plasma AST and ALT levels. *Trans*-resveratrol also did not affect CYP1A1, CYP1A2, CYP2C, or CYP3A activities. In contrast, 0.5% MSE slightly increased CYP1A1 activity. When the mice were fed experimental diets for 12 weeks, 0.05% *trans*-resveratrol increased CYP1A1, CYP2C, and CYP3A activities, whereas 0.5% MSE suppressed CYP3A activity. Under these conditions, 0.5% *trans*-resveratrol enhanced the anticoagulant activity of warfarin, although CYP2C activity increased. However, MSE did not affect the anticoagulant activity of warfarin.**

***Conclusion*: The 0.05% *trans*-resveratrol did not interact with warfarin in a mouse model, whereas 0.5% *trans*-resveratrol may have enhanced the anticoagulant activity of warfarin.**

**Key words:** Cytochrome P-450 (CYP), Dietary supplement, Drug, *Trans*-resveratrol, Warfarin



## Introduction

*Trans*-resveratrol (*trans*-3,4′,5-trihydroxystilbene), which is found in various foods including grapes, cranberries, and peanuts, has become a popular ingredient in dietary supplements in Japan because it has been shown to have antiaging effects in various animal models[1)]. Melinjo (*Gnetum gnemon L.*), a species belonging to the family *Gnetaceae*, is extensively cultivated in Southeast Asia. Its seeds are nutritious and are the main staple food in some areas[2)]. In Japan, melinjo seed extract (MSE) is also used as an ingredient in dietary supplements. Melinjo seeds are very rich in resveratrol dimers (such as gnetin C and its glucosides, gnemonoside A, and gnemonoside D)[3)] (**Table 1**). However, it is reported that dimers of resveratrol show different properties[4)] and effects[5)] to those of *trans*-resveratrol.

The population of Japan is rapidly aging, and as a result, chronic diseases associated with aging, such as diabetes mellitus, cardiovascular disease, hypertension, osteoporosis, and cancer, have become a widely recognized social issue. Against this background, an increase in health consciousness has prompted people to use dietary supplements to maintain health and prevent diseases. In addition to its antiaging effects, resveratrol has been suggested to be effective against obesity, dia-

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betes mellitus[6], atherosclerosis[7, 8], hypertension[9], Alzheimer's disease[10], and cancers[11].

The concurrent use of dietary supplements and drugs has recently become prevalent[12]. Physicians and pharmacists need to be aware of which of their patients are concomitantly using dietary supplements and medicines because many herbs (e.g., black cohosh, coleus forskohlii, echinacea, garlic, ginkgo, ginseng, green tea, kava, milk thistle, and St. John's wort)[13-18] and ingredients (e.g., catechins[19], curcuminoids[20], isoflavones[21], quercetin[22], and polyphenols[23]) affect drug-metabolizing enzymes. Resveratrol[24] also affects the drug-metabolizing enzyme cytochrome P-450 (CYP). Resveratrol was initially found to inhibit CYP1A1 in human HepG2 hepatoma cells[25], with inhibition of human recombinant CYP1B1[26], CYP3A4, CYP3A5[27], and others subsequently being reported. Resveratrol was also shown to inhibit the enzyme activities of CYP2D6, CYP2C9, and CYP3A4 in a healthy volunteer[28]. In contrast, the influence of MSE on drug-metabolizing enzymes has not yet been examined.

The ingredients in dietary supplements have to be absorbed and exist in their active forms to interact with drugs. Some ingredients are not absorbed or are metabolized immediately after their absorption. The concentrations of these ingredients at the sites of interactions are also an important factor. Ingredients have to exist at an effective dose in the blood or target organs to interact with drugs. Furthermore, even if dietary supplements affect drug metabolism and blood levels, drug efficacy is not affected if the drug exists at a therapeutic dose in the blood. Therefore, not only the ingredients but also their concentrations and duration of intake are important.

In the present study, we examined the effects of the intake of several amounts of *trans*-resveratrol and MSE for short and long periods on major CYP subtypes such as Cyp1a1, 1a2, 2c29, and 3a11 in a mouse model (human homologues Cyp1a1, 1a2, 2c9, and 3a4, respectively) as well as the interaction between *trans*-resveratrol or MSE and warfarin *in vivo*.

## Materials and Methods

### Animals

Male C57BL/6J mice were purchased from Japan SLC Inc. (Shizuoka, Japan) and were maintained under specific pathogen-free conditions in a temperature-controlled room (22°C ± 2°C) with a 12-h light/dark cycle. Animals had free access to a normal laboratory diet (CRF-1) and water until 10 weeks of age, when experiments were initiated. The mice were fed an AIN-93 semi-purified diet or diet containing several doses of *trans*-resveratrol or MSE for 1 or 12 weeks.

**Table 1.** Composition of the melinjo seed extract (MSE)

| Components | /100 g MSE |
|---|---|
| Nutrients | |
| Moisture | 4.8 g |
| Protein | 9.3 g |
| Fat | 0.9 g |
| Ash | 5.2 g |
| Available carbohydrate | 79.8 g |
| Dietary fiber | < 0.5 g |
| Sodium | 5.0 mg |
| Phosphorus | 205 mg |
| Iron | 0.33 mg |
| Calcium | 10.9 mg |
| Potassium | 2.44 g |
| Magnesium | 167 mg |
| Copper | 2.59 mg |
| Zinc | 0.60 mg |
| Manganese | 1.07 mg |
| Selenium | 9 mg |
| Chlorine | 179 mg |
| *Trans*-resveratrol and its derivatives | |
| *trans*-resveratrol | 0.11 g |
| Gnetin C | 2.84 g |
| Gnemonoside A | 13.68 g |
| Gnemonoside D | 4.95 g |
| Gnemonoside C | 1.98 g |
| Gnetin L | 0.25 g |
| trans-piceid | 0.55 g |
| Isorhapontigenin | 0.20 g |

The composition of MSE was shown in **Table 1**[29]. To evaluate the interaction between *trans*-resveratrol or MSE and drugs, the mice were fed an AIN-93 semi-purified diet or diet containing several doses of *trans*-resveratrol or MSE for 12 weeks and then administered warfarin racemate (A2250; Sigma-Aldrich Inc., St Louis, MO) (0.33 mg/kg) dissolved in 0.5% carboxymethylcellulose or vehicle via an intragastric injection for the last 2 days of the treatment regimen. Each group comprised five mice. All animal experiments were conducted with the approval of the National Institute of Health and Nutrition Laboratory Animal Ethics Committee.

### Determination of *Trans*-Resveratrol or MSE doses in Diets

The diets used in this experiment were AIN-93 semi-purified diets containing 0.005%, 0.05%, or 0.5% *trans*-resveratrol or MSE. According to the Japan Health and Nutrition Food Association, the recommended daily doses of total resveratrol in dietary supplements are 2−100 mg/day (approximately 0.033−

**Table 2.** Body weight, liver weight, and liver functional markers in plasma with 1 week of feeding

| | | Control | 0.005% | 0.05% | 0.5% |
|---|---|---|---|---|---|
| Body weight (g) | Res | 20.8 ± 0.4 | 20.8 ± 0.4 | 20.8 ± 0.4 | 20.5 ± 0.1 |
| | MSE | 21.5 ± 0.4 | 21.2 ± 0.4 | 21.4 ± 0.2 | 21.6 ± 0.4 |
| Liver weight (g) | Res | 0.83 ± 0.01 | 0.81 ± 0.02 | 0.84 ± 0.02 | 0.81 ± 0.03 |
| | MSE | 0.86 ± 0.02 | 0.85 ± 0.02 | 0.83 ± 0.01 | 0.81 ± 0.01 |
| AST (IU/L) | Res | 12.4 ± 0.6 | 14.6 ± 1.1 | 14.2 ± 1.9 | 15.3 ± 0.6 |
| | MSE | 12.1 ± 0.4 | 13.5 ± 0.5 | 15.6 ± 2.4 | 13.2 ± 0.4 |
| ALT (IU/L) | Res | 3.7 ± 0.2 | 5.0 ± 0.8 | 3.8 ± 0.2 | 4.7 ± 0.1 |
| | MSE | 3.6 ± 0.3 | 4.0 ± 0.4 | 4.4 ± 0.3 | 4.5 ± 0.4 |
| ALP (IU/L) | Res | 64.1 ± 1.5 | 65.7 ± 4.5 | 72.9 ± 2.1 | 68.5 ± 3.3 |
| | MSE | 63.9 ± 1.3 | 72.2 ± 4.3 | 70.0 ± 1.4 | 66.8 ± 0.9 |

Data presented as the mean ± SEM. Body weight, liver weight, and plasma levels of AST, ALT, and ALP were measured 1 week after the initiation of the experimental diet. Res; resveratrol, MSE; melinjo seed extract. $n = 5$ in each group.

1.7 mg/kg BW) for humans. MSE, which we used in this study, contains approximately 25% total resveratrol (*trans*-resveratrol and its derivatives), and then the recommended daily doses of MSE in dietary supplements are 8−400 mg/day (approximately 0.13−6.7 mg/kg BW). This dose translated to 0.4−20 mg/kg BW of resveratrol or 1.6−80 mg/kg BW of MSE for mice using the body surface area normalization method[30]. Because mice (25 g BW) consume approximately 4 g diet/day, 0.05% (w/w) in the diet is similar to 80 mg/kg BW intake. Therefore, we examined the effects of 0.005%, 0.05%, or 0.5% *trans*-resveratrol or MSE in the diet.

**Plasma Chemistry**

After 1 or 12 weeks of feeding, the mice were killed under isoflurane anesthesia after overnight fasting. Blood samples were obtained from the abdominal aorta, and plasma samples were prepared immediately. Plasma levels of AST (transaminase CII-test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan), ALT (transaminase CII-test Wako, Wako Pure Chemical Industries, Ltd.), and ALP (LabAssay ALP, Wako Pure Chemical Industries, Ltd.) were determined with enzymatic methods, respectively.

**Quantitative RT-PCR**

After 1 or 12 weeks of feeding, total RNA was extracted from the liver using the TRIzol Plus RNA Purification System (Life Technologies, Carlsbad, CA) and reverse transcribed with PrimeScript RT Master Mix (Takara Bio Inc., Shiga, Japan). Quantitative RT-PCR was performed on 96-well plates with the SYBR Green PCR Master Mix and Thermal Cycler Dice Real Time System Single (Takara Bio Inc.). Results were expressed as the copy number ratio of the target mRNA to Gapdh mRNA. The following mouse-specific primer pairs were used:
Gapdh forward 5′-TGATGCTGGTGCTGAGTATGTCGT-3′;
reverse 5′-TCTCGTGGTTCACACCCATCACAA-3′;
Cyp1a1 forward 5′-AGCTTGGCCTGGATTACTGT-3′;
reverse 5′-AACCCCATCAACCCCAGTAG-3′;
Cyp1a2 forward 5′-ACATCACAAGTGCCCTGTTCAAGC-3′;
reverse 5′-ATCTTCCTCTGCACGTTAGGCCAT-3′;
Cyp2c29 forward 5′-AGCCTACTGTCATATTGCACGGGT-3′;
reverse 5′-CATGCCCAAATTTCGCAGGGTCAT-3′;
Cyp3a11 forward 5′-AGGCAGAAGGCAAAGAAAGGCAAG-3′;
reverse 5′-TGAGGGAATCCACGTTCACTCCAA-3′;

**Preparation of the Liver Microsomal Fraction**

Livers were homogenized in 50 mM Tris-HCl buffer containing 0.25 M sucrose (pH7.4) with a polytron homogenizer. The homogenate was centrifuged at 10,000 ×*g* for 30 min at 4°C, and the supernatant was collected. The supernatant was re-centrifuged at 105,000 ×*g* for 60 min at 4°C, and the supernatant was discarded. The pellet was re-suspended in 50 mM Tris-HCl buffer (pH7.4) and used as the liver microsomal fraction. Protein concentrations were determined using the BCA protein assay kit (Pierce, Rockford, IL).

**Measurement of CYP Activity**

The activity of each CYP subtype in the liver microsomal fraction was measured using a luminescent method using the P450-Glo™ CYP1A1 System (Luciferin-CEE) Assay, CYP1A2 System (Luciferin-

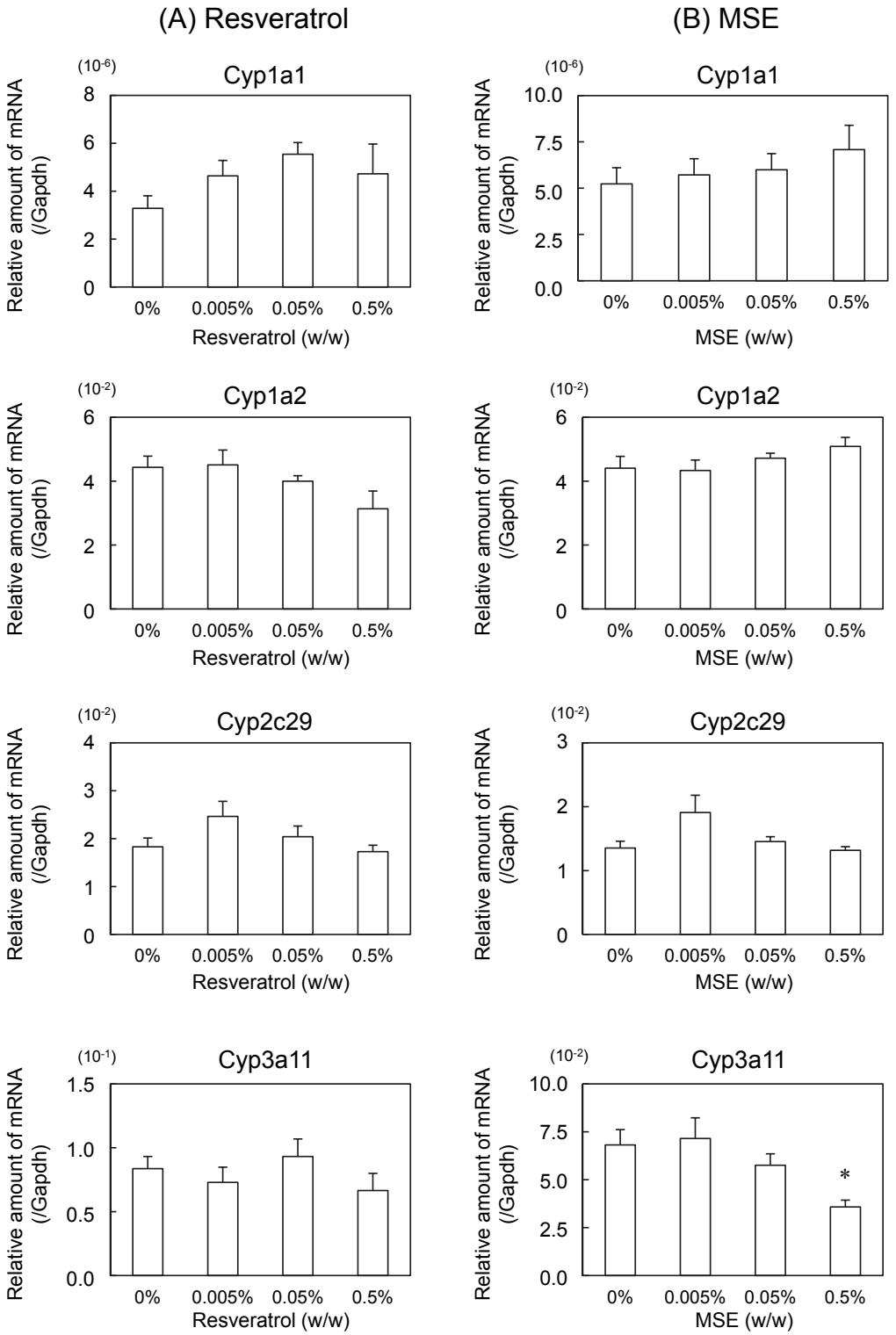


**Fig. 1.** Effects of *trans*-resveratrol and MSE on CYP mRNA expression (1 week of feeding). C57BL/6J mice (male, 10 weeks of age) were fed experimental diets containing 0%, 0.005%, 0.05%, or 0.5% (w/w) *trans*-resveratrol (A) or MSE (B) for 1 week. After overnight fasting, the mice were killed, and the mRNA expression levels of the major CYP subtypes in the liver were measured using real-time qPCR methods. Data are presented as a percentage of the control. Bars show SEM. $^{*}P<0.05$ vs. 0% MSE by a one-way ANOVA with a Bonferroni post hoc test. $n=5$ in each group.

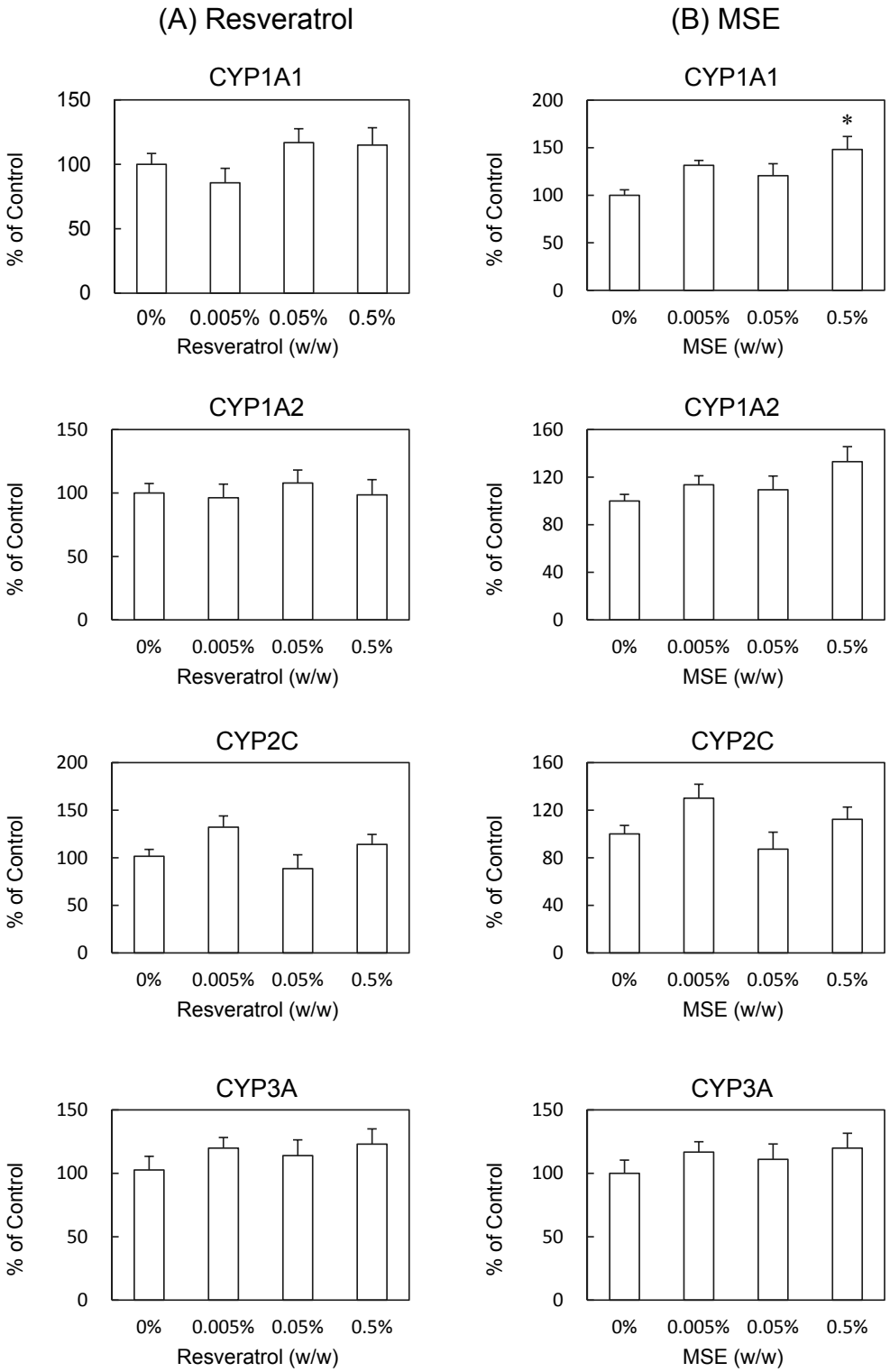


**Fig. 2.** Effects of *trans*-resveratrol and MSE on CYP subtype activities (1 week of feeding). C57BL/6J mice were fed experimental diets containing 0%, 0.005%, 0.05%, or 0.5% (w/w) *trans*-resveratrol (A) or MSE (B) for 1 week. Liver microsomal CYP activities were measured. Data are presented as a percentage of the control. Bars show SEM. *$P<0.05$ vs. 0% MSE by a one-way ANOVA with a Bonferroni post hoc test. $n=5$ in each group.

**Table 3.** Body weight, liver weight, liver functional markers in plasma with 12 weeks of feeding

| | Control | Resveratrol | | Melinjo seed extract | |
|---|---|---|---|---|---|
| | | 0.05% | 0.5% | 0.05% | 0.5% |
| Body weight (g) | 36.5 ± 0.9 | 34.0 ± 1.3 | 34.7 ± 1.7 | 35.6 ± 1.5 | 34.2 ± 0.6 |
| Liver weight (g) | 1.16 ± 0.03 | 1.10 ± 0.04 | 1.11 ± 0.05 | 1.08 ± 0.06 | 0.97 ± 0.01* |
| Liver functional markers | | | | | |
| AST (IU/L) | 23.4 ± 4.6 | 13.7 ± 0.8* | 19.9 ± 1.3 | 16.2 ± 2.1 | 13.5 ± 0.7 |
| ALT (IU/L) | 1.8 ± 0.2 | 1.4 ± 0.6 | 1.9 ± 0.3 | 1.8 ± 0.5 | 1.4 ± 0.3 |
| ALP (IU/L) | 42.1 ± 2.1 | 51.6 ± 2.0* | 44.7 ± 2.6 | 39.1 ± 0.9 | 39.5 ± 1.9 |

Data presented as the mean ± SEM. Body weight, liver weight, and plasma levels of AST, ALT, and ALP were measured 12 weeks after the initiation of the experimental diet. *$P < 0.05$ vs. the control by a one-way ANOVA with a Bonferroni post hoc test. $n = 5$ in each group.

1A2) Assay, CYP2C9 System (Luciferin-H) Assay, CYP3A4 System (Luciferin-PPXE) Assay, and NADPH Regeneration System with GloMax-Multi+ Detection System (Promega Co., Madison, WI). CYP activity was adjusted by protein concentrations, and results were represented as a percentage of the control.

### Measurement of Anticoagulant Activity

Blood samples were immediately centrifuged at 800 ×*g* at 4°C for 15 min to prepare plasma. Coagulation parameters [prothrombin time (PT), activated partial thromboplastin time (APTT), and thrombotest Owren (TTO)] were measured using an automated blood coagulation analyzer (CA-50; Sysmex Co., Hyogo, Japan) according to the manufacturer's protocol. PT and TTO are indicators of the extrinsic and common pathways of the coagulation cascade, respectively, and are used to monitor warfarin therapy. APTT is an indicator of both the intrinsic and common pathways of the coagulation cascade.

### Statistical Analysis

Data are presented as the mean ± SEM. Comparisons of data from multiple groups were performed by a one-way ANOVA with a Bonferroni post hoc test (SPSS 18.0J for Windows, IBM Co. Armonk, NY). $P < 0.05$ was considered to be significant.

## Results

### Effects of *Trans*-Resveratrol and MSE for a Short Period (1 Week of Feeding)

When the mice were fed experimental diets containing 0.005%, 0.05%, or 0.5% *trans*-resveratrol or MSE for 1 week, neither *trans*-resveratrol nor MSE affected the body or liver weights (**Table 2**). Moreover, neither *trans*-resveratrol nor MSE affected the plasma levels of AST, ALT, or ALP (**Table 2**). Under these conditions, *trans*-resveratrol did not affect Cyp1a1, 1a2, 2c29, or 3a11 mRNA expression levels in the liver (**Fig. 1A**) or their activities in liver microsomal fractions (**Fig. 2A**). In contrast, 0.5% MSE significantly suppressed Cyp3a11 mRNA expression levels and increased CYP1A1 activity, whereas 0.005% and 0.05% MSE did not (**Fig. 1B** and **2B**).

### Effects of *Trans*-Resveratrol and MSE for a Long Period (12 Weeks of Feeding)

We examined the effects of 12 weeks of feeding diets with 0.05% or 0.5% *trans*-resveratrol and MSE. When the mice were fed experimental diets for 12 weeks, *trans*-resveratrol and MSE slightly suppressed body weight gain (**Table 3**). In addition, liver weights were significantly lower in mice that were fed the 0.5% MSE diet than in control mice (**Table 3**). *Trans*-resveratrol and MSE suppressed the plasma levels of AST but did not affect those of ALT. In contrast, 0.05% *trans*-resveratrol significantly increased the plasma levels of ALP but within normal ranges. Under these conditions, 0.05% *trans*-resveratrol slightly increased Cyp3a11 mRNA expression levels in the liver (**Fig. 3**) and significantly increased CYP1A1, 2C, and 3A activities in liver microsomal fractions (**Fig. 4**), whereas 0.5% *trans*-resveratrol did not. In contrast, MSE did not affect CYP mRNA expression levels (**Fig. 3**), whereas 0.5% MSE significantly suppressed CYP3A activity (**Fig. 4**).

### Interaction of *Trans*-Resveratrol or MSE with Warfarin

*Trans*-resveratrol (0.05%, 12 weeks) significantly increased the activities of the major CYPs, including CYP2C, and may have influenced the efficacy of the drugs metabolized by CYP2C. To address this issue, the mice were fed an experimental diet containing 0.05% or 0.5% *trans*-resveratrol or MSE for 12 weeks, and warfarin racemate or vehicle was then administrated on the last 2 days. The co-administration of

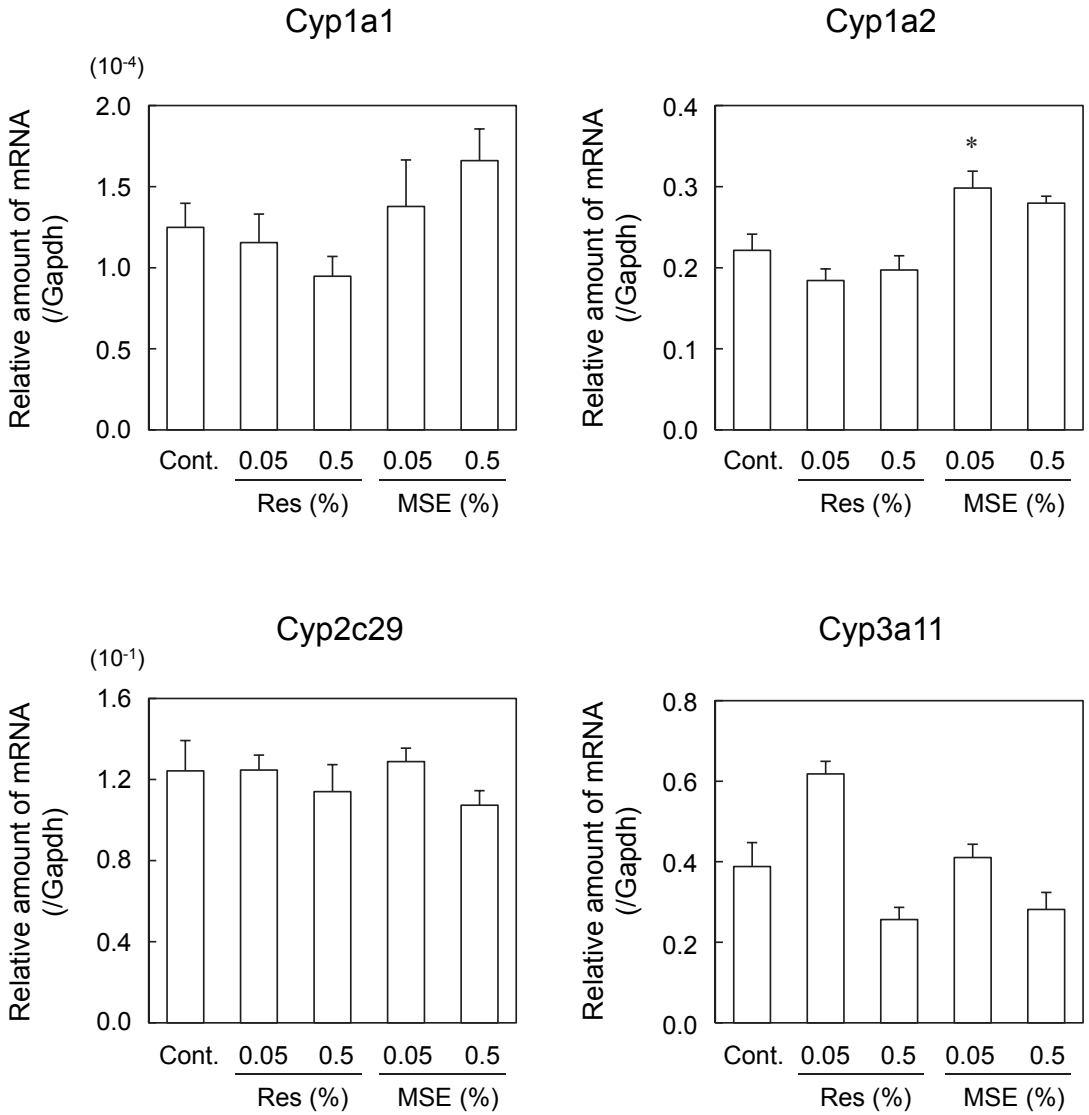


**Fig. 3.** Effects of *trans*-resveratrol and MSE on CYP mRNA expression levels (12 weeks of feeding). C57BL/6J mice were fed experimental diets containing 0%, 0.05%, or 0.5% (w/w) *trans*-resveratrol or MSE for 12 weeks. The major CYP subtype mRNA expression levels in the liver were measured using real-time qPCR methods. Data are presented as a percentage of the control. Bars show SEM. *$P<0.05$ vs. 0% MSE by a one-way ANOVA with a Bonferroni post hoc test. $n=5$ in each group.

*trans*-resveratrol and warfarin did not affect the body and liver weights (**Table 4**). Under these conditions, *trans*-resveratrol itself did not affect PT, APTT, or TTO (**Fig. 5A**). However, in the presence of warfarin, PT and APTT were higher with 0.5% *trans*-resveratrol than with 0% *trans*-resveratrol (**Fig. 5A**). In contrast, MSE slightly decreased the body and liver weights with or without warfarin (**Table 4**). Under these conditions, 0.5% MSE itself slightly shortened APTT (**Fig. 5B**). In the presence of warfarin, PT, APTT, and TTO were slightly higher with 0.5% MSE than with 0% MSE, but it was not statistically significance (**Fig. 5B**).

## Discussion

In the present study, 0.05% *trans*-resveratrol in the diet enhanced the activities of CYP1A1, 2C, and 3A but did not affect the anticoagulant activity of warfarin. In contrast, 0.5% *trans*-resveratrol in the diet did not affect the activities of CYP, but enhanced the anticoagulant activity of warfarin, even though *trans*-

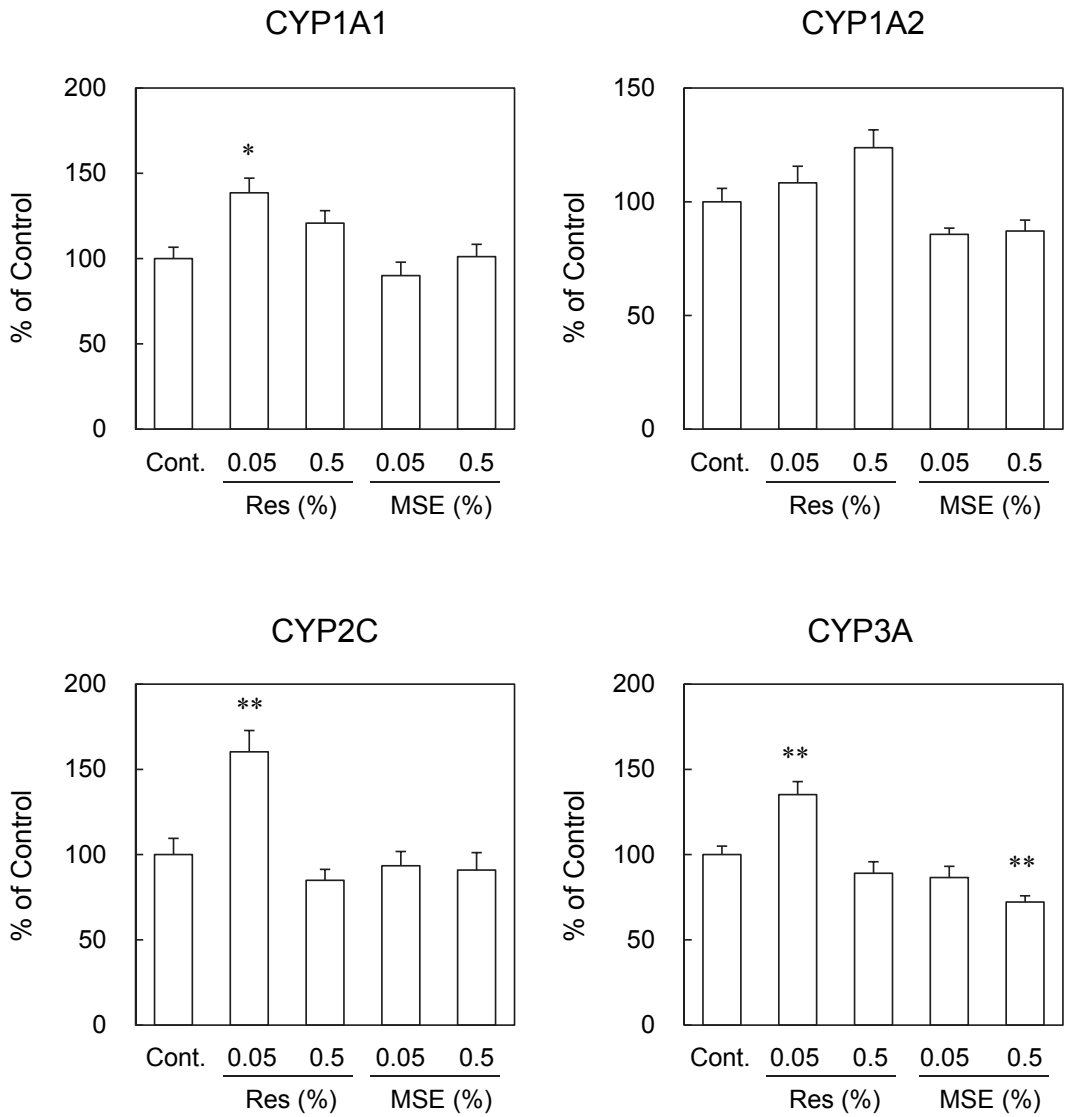


**Fig. 4.** Effects of *trans*-resveratrol and MSE on CYP subtype activities (12 weeks of feeding). C57BL/6J mice were fed experimental diets containing 0%, 0.05%, or 0.5% (w/w) *trans*-resveratrol or MSE for 12 weeks. Liver microsomal CYP activities were measured. Data are presented as a percentage of the control. Bars show SEM. $^{*}P<0.05$, $^{**}P<0.01$ vs. control by a one-way ANOVA with a Bonferroni post hoc test. $n=5$ in each group.

resveratrol itself did not exhibit anticoagulant activity. MSE did not affect the anticoagulant activity of warfarin.

Two possibilities have been suggested for why *trans*-resveratrol enhanced the anticoagulant activity of warfarin, whereas MSE did not in this experiment. MSE is mainly composed of resveratrol derivatives, the characteristics of which differ from those of *trans*-resveratrol[4, 5]. Therefore, the abilities of *trans*-resveratrol and MSE (the mixture of active compounds, which including *trans*-resveratrol and resveratrol derivatives) to interact with drugs differ. The second possibility, MSE contains approximately 25% of *trans*-resveratrol and its derivatives, indicating that 0.5% MSE in diet is similar to 0.125% active compounds in diet. In the present study, 0.5% MSE slightly, but not significantly, increased PT, APTT, and TTO. Therefore, there is a possibility that more than 0.5% of MSE in diet may enhance the anticoagulant activity of warfarin. However, when people use MSE dietary supplements within the recommended daily dose, MSE could not interact with warfarin.

We previously surveyed dietary supplement usage in patients in Japan and found that some had been

**Table 4.** Body weight and liver weight with 12 weeks of feeding

| | Control | | | Warfarin | | |
|---|---|---|---|---|---|---|
| | 0% | 0.05% | 0.5% | 0% | 0.05% | 0.5% |
| Resveratrol | | | | | | |
| Body weight (g) | 28.4 ± 0.5 | 31.6 ± 0.7* | 30.4 ± 0.9 | 30.8 ± 0.6 | 31.5 ± 0.9 | 29.8 ± 0.5 |
| Liver weight (g) | 1.09 ± 0.03 | 1.08 ± 0.04 | 1.04 ± 0.06 | 1.10 ± 0.03 | 1.13 ± 0.04 | 0.99 ± 0.06 |
| MSE | | | | | | |
| Body weight (g) | 35.9 ± 1.2 | 33.2 ± 1.5 | 33.4 ± 2.5 | 34.7 ± 1.1 | 30.8 ± 0.8* | 31.2 ± 0.4 |
| Liver weight (g) | 1.07 ± 0.05 | 0.99 ± 0.05 | 1.01 ± 0.07 | 1.07 ± 0.04 | 0.97 ± 0.02 | 0.98 ± 0.02 |

Data presented as the mean ± SEM. Body and liver weights were measured 12 weeks after the initiation of the experimental diet. *$P < 0.05$ vs. 0% resveratrol or MSE in each treatment by a one-way ANOVA with a Bonferroni post hoc test. $n = 4$ or 5 in each group.

concomitantly taking dietary supplements and medicines[12]. However, approximately 70% of these patients did not declare the use of dietary supplements to their physicians[12]. Therefore, health issues caused by interactions between dietary supplements and drugs may occur. Interactions between dietary supplements and drugs have mainly been observed at the absorption and metabolism steps[31]. ATP-binding cassette (ABC) transporters are associated with the absorption of drugs in the intestines. ABCB1, also known as P-glycoprotein, has been shown to play an important role in drug efflux[32]. Previous studies showed that resveratrol inhibited P-glycoprotein activity both *in vitro*[33] and *in vivo*[34] models. However, it is reported that the absorption of warfarin was independent of P-glycoprotein activity[35]. In contrast, CYPs are known to play an important role in drug metabolism. The effects of resveratrol on CYP1A1, 1A2, 1B1, and 3A4 have been extensively investigated[24], whereas few studies have examined CYP2C. In the present study, *trans*-resveratrol did not affect or increase CYP2C activity, which depended on the time and dose administered. In this study, we used warfarin racemate, and the anticoagulant property of R-warfarin is less than that of S-warfarin[36]. S-warfarin is metabolized by CYP2C9, whereas R-warfarin is metabolized by CYP1A1, 1A2, and 3A4 in humans[36, 37]. In the present study, the activities of CYP1A1, 2C, and 3A were increased by 0.05% resveratrol. Therefore, the metabolism of S-warfarin and R-warfarin may have been enhanced. However, 0.05% resveratrol did not affect the anticoagulant activity of warfarin, whereas 0.5% resveratrol increased it (prolonged PT and APTT), even though the activities of CYPs did not change by 0.5% resveratrol. A previous study reported that *trans*-resveratrol was metabolized by CYP1A1, 1A2, and 1B1[38]. In contrast, it was also reported that *trans*-resveratrol inhibited CYP1A1, 1B1, and 1A2[39]. Previous studies showed that resveratrol suppressed platelet aggregation[40, 41] and prevented atherosclerosis by inhibiting platelet aggregation in a mouse model[42]. This antiplatelet activity of resveratrol may have had an impact on the results obtained in the present study.

Resveratrol is regarded as a candidate for disease therapies against obesity, diabetes mellitus[6], atherosclerosis[7, 8], Alzheimer's disease[10], and cancers[11]. In addition to animal studies, clinical studies have also been conducted. Resveratrol was found to improve insulin sensitivity and postprandial plasma glucose in subjects with impaired glucose tolerance[43]. Its administration was also reported to significantly improve mean hemoglobin A1c, systolic blood pressure, and total cholesterol levels in patients with type 2 diabetes mellitus[44, 45]. Furthermore, a resveratrol-enriched grape extract treatment significantly improved the lipid profile[46] and inflammatory cytokine levels[47] in the blood. Resveratrol has also been shown to induce dose-dependent increases in cerebral blood flow without influencing cognitive function[48]. A previous study reported that it reduced tumor cell proliferation by 5% in patients with colorectal cancer[49]. In contrast, few studies have examined MSE, with only two demonstrating its safety[29, 50].

## Conclusion

In the present study, 0.05% *trans*-resveratrol or MSE did not interact with warfarin, whereas 0.5% *trans*-resveratrol enhanced the anticoagulant effects of warfarin in a mouse model. Although we used a mouse model, our results indicate that care is needed regarding the concomitant use of resveratrol and drugs by patients.

## Acknowledgments

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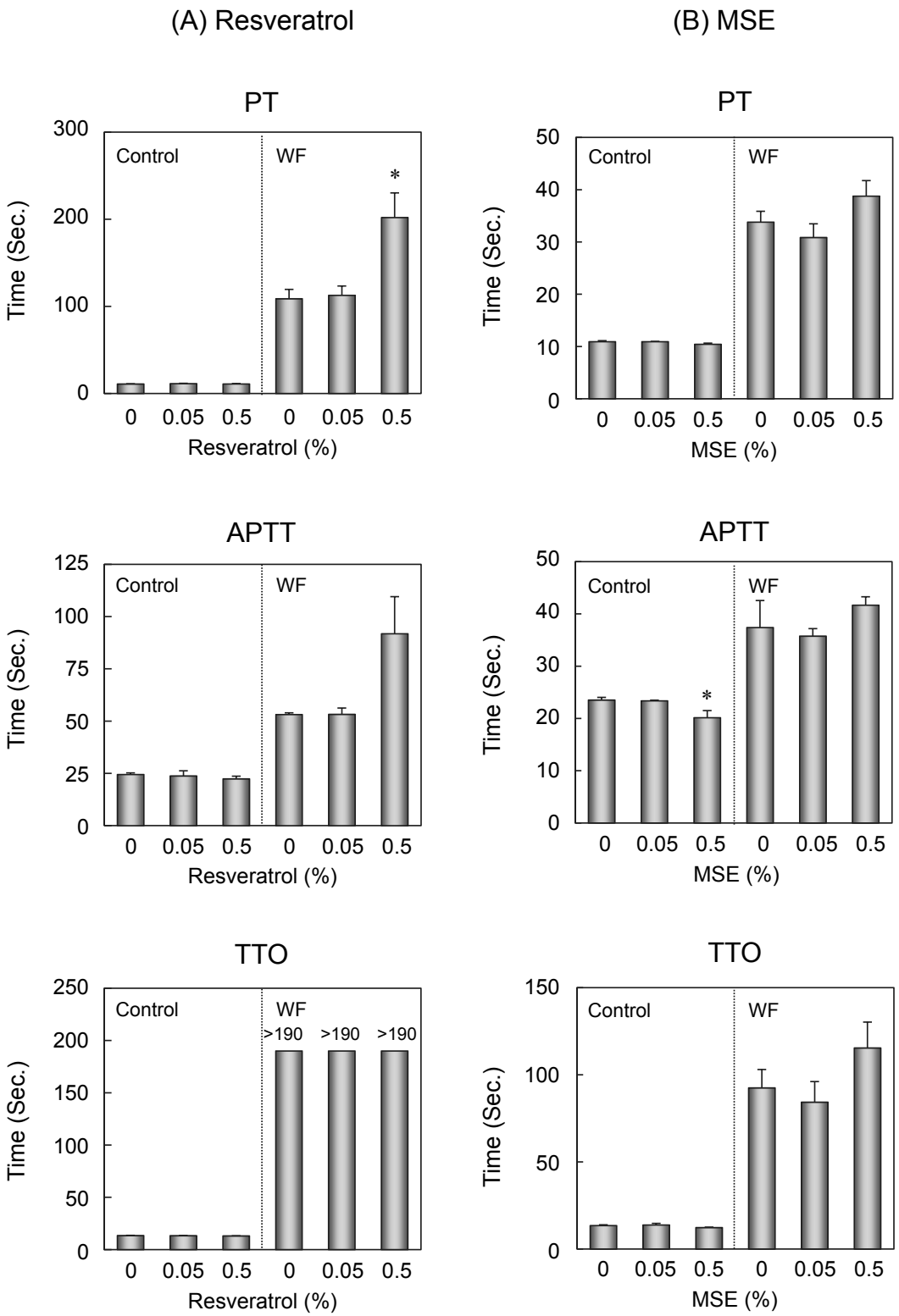


**Fig. 5.** Effects of *trans*-resveratrol and MSE on anticoagulant activities of warfarin (12 weeks of feeding). C57BL/6J mice were fed experimental diets containing 0%, 0.05%, or 0.5% (w/w) *trans*-resveratrol (A) or MSE (B) for 12 weeks. Warfarin racemate (0.33 mg/kg) dissolved in 0.5% carboxymethylcellulose or vehicle was administrated via an intragastric injection for the last 2 days of the treatment regimen. After overnight fasting, the mice were killed, and PT, APTT, and TTO were measured. Bars show SEM. *$P<0.05$ vs. 0% *trans*-resveratrol or MSE in each treatment by a one-way ANOVA with a Bonferroni post hoc test. $n=4$ or 5 in each group.

## Conflicts of Interest

This research was supported by Yamada Bee Company Inc. (Okayama, Japan). Y. Kimura and T. Tatefuji are employees of Yamada Bee Company, Inc. No other authors declare any potential conflicts of interest.

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