Asian Pac J Trop Biomed 2016; 6(9): 778–784

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Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2016.07.009

Vasoprotective effects of rice bran water extract on rats fed with high-fat diet

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ARTICLE INFO

Article history:
Received 21 Dec 2015
Received in revised form 25 Jan 2016
Accepted 2 Mar 2016
Available online 30 Jul 2016

Keywords:
Rice bran
Metabolic syndrome
Cardiovascular disease
Endothelial nitric oxide synthase
Nuclear factor-kappa B
Cluster of differentiation 36

ABSTRACT

Objective: To elucidate the protective effects of rice bran water extract on the expression of endothelial nitric oxide synthase (eNOS), nuclear factor-kappa B (NF-κB), and a cluster of differentiation 36 (CD36) in the vasculature of high-fat diet-fed rats.

Methods: Male Sprague-Dawley rats were divided into three groups. Group I served as control, Group II was treated with high-fat diet, and Group III was treated with high-fat diet and rice bran water extract at 2205 mg/kg/day. After four weeks, the metabolic parameters, malondialdehyde as a marker of oxidative stress, and histological features of the aorta were evaluated. The levels of transcripts and proteins in aorta were determined by real-time PCR and Western blot analysis, respectively.

Results: In comparison with the Group II, rice bran water extract administration resulted in a significant reduction in body weight, visceral fat tissue weights, blood glucose levels, and serum total-cholesterol and free fatty acid levels in Group III. Serum triglyceride levels tended to decrease in the Group III. Also, rice bran water extract administration obviously decreased malondialdehyde levels in both serum and aorta. Interestingly, rice bran water extract treatment demonstrated a significant up-regulation of eNOS expression and down-regulation of NF-κB p65 and CD36 expressions. Nonetheless, all groups showed normal histology of aorta.

Conclusions: Rice bran water extract exhibited vasoprotective effects in the high-fat diet-induced obesity condition by modulating the expression of eNOS, NF-κB, and CD36 and metabolic parameters.

1. Introduction

Metabolic syndrome is a common cluster of metabolic disturbances prevalent worldwide [1]. It is composed of several vascular risk factors including abdominal (visceral) obesity, dyslipidemia, hyperglycemia, and hypertension. In addition, oxidative stress is one of the important pathomechanisms that have been suggested to play a role in the development of metabolic syndrome, coronary artery disease, and hypertension [2]. The excess of reactive oxygen species can induce oxidative damage to biomolecules (*e.g.*, lipids, proteins, and nucleic acids). Among the various lipid oxidative damages, malondialdehyde (MDA) is a key end-product of lipid peroxidation and serves as a biomarker of oxidative stress in an animal model of atherosclerosis and patients with cardiovascular disease [3,4].

Although the pathogenesis of vascular disease in metabolic syndrome is complex, the decreased nitric oxide (NO), enhanced inflammatory responses, and lipid accumulation are involved in the early stages of vascular disease. Endothelial nitric oxide synthase (eNOS), nuclear factor-kappa B (NF-κB), and a cluster of differentiation 36 (CD36) play major roles in vascular NO synthesis,

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All experimental procedures involving animals were conducted in accordance to Association for Assessment and Accreditation of Laboratory Animal Care and approved by the Animal Ethics Committee of the Faculty of Medicine, Thammasat University, Pathum Thani, Thailand (AE 002/2013).

Foundation Project: Supported by Research Unit, Faculty of Medicine, Thammasat University and the Higher Education Research Promotion and National Research University Project of Thailand (Grant No. 2-17/2013 and 2014-76, respectively).

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

inflammation, and lipid uptake, respectively [5–7]. At the molecular level, both animal and human studies have proposed that down-regulation of eNOS expression, as well as up-regulation of NF-κB p65 subunit (NF-κB p65) and CD36 expressions are significant factors of vascular disease [8–13]. Moreover, overexpression of eNOS and inhibition of CD36 and NF-κB signaling have been revealed to protect rodents from the development of vascular disease [14–16]. Thus, the regulation of eNOS, NF-κB, and CD36 expressions in the vasculature is considered to be an important preventive mechanism for cardiovascular disease.

Consumption of rice bran has been shown to be associated with beneficial effects on metabolic and cardiovascular diseases [17,18]. Rice bran contains several nutrients and phytochemicals such as carbohydrates, proteins, fibers, polyphenols, and γ-oryzanols [17]. Our preliminary study demonstrated that rice bran water extract from the Khao Dawk Mali 105 rice variety (*Oryza sativa* Linn.) significantly reduced insulin resistance, as well as abdominal and hepatic fat deposition in rats fed with a high-fat diet for four weeks. Although our results suggested that Khao Dawk Mali 105 rice bran water extract could reduce the vascular risk factors, vasoprotection at the molecular level has not been elucidated yet. Thus, the present study aimed at verifying the effects of rice bran water extract on the expressions of eNOS, NF-κB, and CD36 in the vasculature of high-fat diet-fed rats so as to provide a model for prevention of metabolic syndrome.

2. Materials and methods

2.1. Preparation and characterization of rice bran water extract

The bran of Khao Dawk Mali 105 rice variety was purchased from the local mill in Surin Province, Thailand. Rice was grown in the organic farm approved by the Organic Agriculture Certification of the Department of Agricultural Extension (Bangkok, Thailand). Freshly milled rice bran was stabilized at 130 °C for 90 s. About 2000 g of stabilized rice bran was boiled in 8000 mL of distilled water for 1 h at 70 °C. After centrifugation at 8000 r/min for 10 min, the supernatant was freeze-dried into powdered extract by using a freeze dryer (Lyophilization Systems Inc., USA). The procedure of preparation was described in details by Qureshi *et al.* [17]. The proximate analysis, total phenolic compounds, and γ-oryzanol contents of rice bran water extract were also determined using the official method of Association of Official Analytical Chemists, Folin–Ciocalteu method, and high-performance liquid chromatography [19], respectively.

2.2. Experimental diets and animals

The standard chow (C.P. mice feed, Thailand) consisted of 13%, 55%, and 32% of total energy derived from fat, carbohydrate, and protein, respectively. The high-fat diet was modified from the diet that induces obesity in which 65% of total energy was derived from fat [20]. The major ingredients of the high-fat diet included pork belly, pork liver, margarine, sugar, wheat flour, standard chow, and a whole egg (hen). The high-fat diet consisted of 65%, 24%, and 11% of total energy derived from fat, carbohydrate, and protein, respectively.

Male Sprague-Dawley rats (6–8 weeks old and weighting 180–220 g) were obtained from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom, Thailand. All experimental procedures involving animals were conducted in accordance to Association for Assessment and Accreditation of Laboratory Animal Care and approved by the Animal Ethics Committee of the Faculty of Medicine, Thammasat University, Pathum Thani, Thailand (AE 002/2013). Animals were maintained under controlled temperature of (24 ± 1) °C with 60% humidity and a 12 h light and 12 h dark cycle. After a week of acclimatization, the rats were randomly divided into three groups of eight rats each. Rats in Group I were fed with standard chow (control group). Rats in Group II were fed with high-fat diet alone. Rats in Group III were fed with high-fat diet and orally gavaged with a fixed dose of rice bran water extract (2205 mg/kg/day, dissolved in distilled water). Our preliminary work indicated that rice bran water extract at the dose of 2205 mg/kg/day was effective in improving the metabolic disturbances in high-fat diet-fed rats. Thus, this dose was chosen for the present research. Rats in all three groups were fed with water and experimental diets *ad libitum* throughout four weeks of the experiment. Body weight, food intake, and energy intake were measured daily. At the end of treatment, the animals were sacrificed with an overdose of pentobarbital sodium (intraperitoneal injection), and their blood was collected by a cardiac puncture. Tissues were removed, weighed, and properly kept for histological study or biochemical assays.

2.3. Blood biochemical assessment

The blood glucose levels were determined with a glucometer (Accu-Chek Performa, Roche Diagnostics, Switzerland). The concentrations of total-cholesterol (total-C), high-density lipoprotein-cholesterol (HDL-C), and triglyceride (TG) in the serum were analyzed using enzymatic colorimetric method (Fluitest test kits, Analyticon Biotechnologies AG, Germany). The serum low-density lipoprotein-cholesterol (LDL-C) level was determined using the Friedewald equation [21]: LDL-C = Total-C – HDL-C – (TG/5). The concentrations of free fatty acid (FFA) were measured using enzymatic colorimetric method (FFA assay kit, Wako, Japan).

2.4. Measurement of serum and aortic MDA

MDA levels were analyzed as a biomarker for oxidative damage. The concentrations of MDA in the serum and aortic tissues were determined spectrophotometrically at 532 nm according to a previously published method with some modifications [22], using 1,1,3,3-tetraethoxypropane (Sigma-Aldrich, USA) as a standard. For tissue samples, total protein levels were used for normalization of MDA levels and determined by Bradford protein assay kit (Bio-Rad, USA) according to the manufacturer's instructions. Serum and aortic MDA levels were expressed as nmol/dL and nmol/mg of protein, respectively.

2.5. Real-time PCR analysis

Total RNA from the aortas was extracted using TRIzol reagent (Invitrogen, USA), according to the manufacturer's recommendations. Total RNA concentration and purity were determined by the NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). Subsequently, RNA (200 ng) was reverse transcribed into cDNA using the cDNA reverse transcription kit (Applied Biosystems, USA) according to the manufacturer's instructions. Quantitative PCR was performed using the TaqMan reagent kit and StepOnePlus real-time PCR system (Applied Biosystems, USA). The relative mRNA levels of eNOS (assay ID Rn02132634_m1), NF-κB p65 (assay ID Rn01502266_m1), and CD36 (assay ID Rn02115479_g1) were analyzed by the $2^{-\Delta\Delta CT}$ method. The

expression levels of glyceraldehyde 3-phosphate dehydrogenase (assay ID Rn99999916_s1) were used for normalization.

2.6. Western blot analysis

The total protein of the aortic tissue was isolated using the cell lysis buffer (Cell Signaling Technology, USA) according to the manufacturer's instructions. The total protein concentration was determined by Quick Start Bradford protein assay kit (Bio-Rad, USA). Equal amounts of sample proteins (15 μg) were electrophoresed on 10% sodium dodecyl sulfate-polyacrylamide gel and electroblotted onto nitrocellulose membrane (Bio-Rad, USA). The membranes were blocked by a mixture of Odyssey blocking buffer (LI-COR Bioscience, USA) and Tris-buffered saline (1:1). Then, the membranes were incubated with a primary eNOS antibody (1:200), CD36 antibody (1:200) (Santa Cruz Biotechnology, USA), NF-κB p65 antibody (1:500), and β-actin antibody (1:1 000) (Cell Signaling Technology, USA) overnight at 4 °C. The membranes were washed thrice with Tris-buffered saline/0.1% Tween-20 and incubated with a secondary antibody (1:10 000) [anti-rabbit immunoglobulin G (H + L), DyLight 680 conjugate, Cell Signaling Technology, USA] for 1 h at room temperature. After washing, the densities of bands were determined using the Odyssey Fc Imaging System (LI-COR Bioscience, USA). The protein levels of β-actin were used for normalization.

2.7. Hematoxylin and eosin (H & E) staining of tissue sections

Thoracic aortas were fixed in 10% formalin and embedded in paraffin. Tissue sections were subsequently stained with H & E. Images were detected under a light microscope (Eclipse Ci-L microscope, Nikon, Japan) coupled to a digital microscope camera (DS-Fi2 microscope camera, Nikon). The area of the aortic wall was measured for each rat (n = 3 per group) by AxioVision microscopy software (Carl Zeiss, Germany) and calculated as follows: Area of the aortic wall = Cross-sectional area of the whole aorta − Cross-sectional area of the lumen. Aortic tunica media thickness was measured from ten different points of cross section and expressed as an average.

2.8. Statistical analysis

All values were statistically analyzed using SPSS version 16.0 (SPSS Inc., USA) and expressed as mean ± SEM. The results were analyzed using One-way ANOVA, followed by the least significant difference's *post hoc* test. Statistically, the significant difference was considered as $P < 0.05$.

3. Results

3.1. Characterization of rice bran water extract

An approximate 18.3% yield of crude extract was obtained. The proximate analysis revealed that rice bran water extract contained carbohydrate (63.9%), protein (12.9%), fat (1.4%), ash (11.4%), moisture (10.3%), and insoluble dietary fiber (0.7%). The contents of total phenolic compounds and γ-oryzanols were (4.6 ± 0.3) mg gallic acid equivalents/g extract and (4.6 ± 0.1) μg/g extract, respectively.

3.2. Effects of rice bran water extract on dietary intake, body weight, and visceral fat weight

As presented in Table 1, Group I consumed significantly more food than Group II per day ($P < 0.001$). On the other hand, the energy intake was significantly higher in Groups II and III than in Group I ($P < 0.001$). Food and energy intakes were not significantly different between Groups II and III. The initial body weight of rats before treatment did not significantly differ among the experimental groups. After four weeks, body weight, body weight gain, and weight of omental and epididymal fat tissues of rats were significantly increased in Group II compared with Group I ($P < 0.001$). These parameters were significantly lower in Group III than in Group II ($P < 0.001$).

3.3. Effects of rice bran water extract on fasting blood glucose (FBG) and serum lipid profile

Blood biochemical parameters from each group are shown in Table 2. In comparison with Group I, FBG ($P < 0.001$), serum TG ($P < 0.05$), FFA ($P < 0.001$), and LDL-C levels ($P < 0.01$) were elevated, but serum HDL-C levels ($P < 0.05$) were decreased in Group II. Total-C levels in serum also showed a tendency to increase in Group II compared with Group I, but the difference was not statistically significant. Treatment with rice bran water extract caused a significant decrease in FBG ($P < 0.001$), the serum levels of total-C ($P < 0.05$), and FFA ($P < 0.001$) when compared to rats fed with the high-fat diet alone. Serum TG levels also tended to reduce in the rice

Table 1
Dietary intake, body weight, and fat tissue weight of the experimental groups.

Groups	Food (g/day)	Energy (kcal/day)	Initial BW (g)	Final BW (g)	BW gain (g)	Omental fat (%BW)	Epididymal fat (%BW)
Group I	23.2 ± 0.1	70.6 ± 0.2	243.9 ± 2.1	366.2 ± 0.9	123.5 ± 1.4	1.3 ± 0.1	1.5 ± 0.0
Group II	19.4 ± 0.3***	99.5 ± 1.7***	243.6 ± 1.9	412.3 ± 2.8***	169.5 ± 2.3***	2.2 ± 0.1***	2.1 ± 0.1***
Group III	19.1 ± 0.5***	97.6 ± 2.6***	244.0 ± 1.6	368.6 ± 7.4$^{###}$	123.0 ± 5.5$^{###}$	1.5 ± 0.1$^{###}$	1.6 ± 0.1$^{*,###}$

Values are expressed as mean ± SEM (n = 8). *: $P < 0.05$, ***: $P < 0.001$ compared with Group I; $^{###}$: $P < 0.001$ compared with Group II. BW: Body weight.

Table 2
Blood biochemical parameters of the experimental groups.

Groups	FBG (mg/dL)	Total-C (mg/dL)	TG (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	FFA (mg/dL)
Group I	104.9 ± 1.4	88.0 ± 2.3	33.3 ± 3.0	26.0 ± 2.6	53.7 ± 1.1	14.8 ± 0.4
Group II	115.4 ± 0.9***	95.4 ± 3.7	41.7 ± 2.6*	38.5 ± 3.6**	47.1 ± 1.7*	17.6 ± 0.1***
Group III	105.6 ± 1.5$^{###}$	85.0 ± 2.8$^{#}$	35.0 ± 2.2	31.3 ± 1.9**	48.5 ± 3.1	14.3 ± 0.5$^{###}$

Values are expressed as mean ± SEM (n = 8). *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ compared with Group I; $^{#}$: $P < 0.05$, $^{###}$: $P < 0.001$ compared with Group II.

bran water extract-treated rats. However, the serum levels of LDL-C and HDL-C were not significantly improved in Group III.

3.4. Effects of rice bran water extract on serum and aortic MDA levels

Figure 1 shows the effect of rice bran water extract on MDA levels in serum and aorta of experimental animals. After four weeks of feeding, increased serum and aortic MDA levels were found in rats fed with the high-fat diet alone ($P < 0.01$). In contrast, treatment of rats fed the high-fat diet with rice bran water extract showed a significant decrease in the serum and aortic MDA levels ($P < 0.001$).

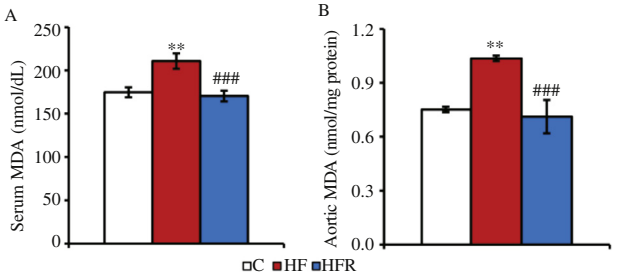

Figure 1. MDA levels in the serum (A) and aortas (B) of the experimental groups.
Values are expressed as mean ± SEM ($n = 8$). **: $P < 0.01$ compared with Group I; ###: $P < 0.001$ compared with Group II. C: Group I; HF: Group II; HFR: Group III.

3.5. Effects of rice bran water extract on eNOS, NF-κB p65, and CD36 expressions and histological features of the aorta

eNOS mRNA expression was significantly lower in Group II than in Group I ($P < 0.01$, Figure 2A). Likewise, eNOS protein levels showed a tendency to decrease in Group II compared with Group I, but the difference was not significant. In contrast, the administration of rice bran water extract in Group III significantly increased the expression of eNOS mRNA and protein when compared to rats fed the high-fat diet alone ($P < 0.001$). Group III also significantly increased expression of eNOS protein when compared to Group I ($P < 0.001$). The NF-κB p65 mRNA and protein expressions were significantly higher in Group II than in Group I ($P < 0.001$ and $P < 0.05$, respectively, Figure 2B). Treatment with rice bran water extract in Group III significantly decreased both NF-κB p65 mRNA and protein expressions as compared with Group II ($P < 0.001$ and $P < 0.01$, respectively). The expressions of CD36 mRNA and protein were significantly increased in the aorta of Group II compared with those of Group I ($P < 0.001$, Figure 2C). Conversely, the expression levels of CD36 were significantly lower in Group III than in Group II ($P < 0.001$) although these levels in Group III were still higher than those in Group I.

As shown in Figure 3, sections from all animals showed the normal structure of the aorta and no evidence of atherosclerotic lesions (Figure 3A). Furthermore, there was no significant difference in the area of the aortic wall and the medial thickness of the aorta among the experimental groups (Figure 3B,C).

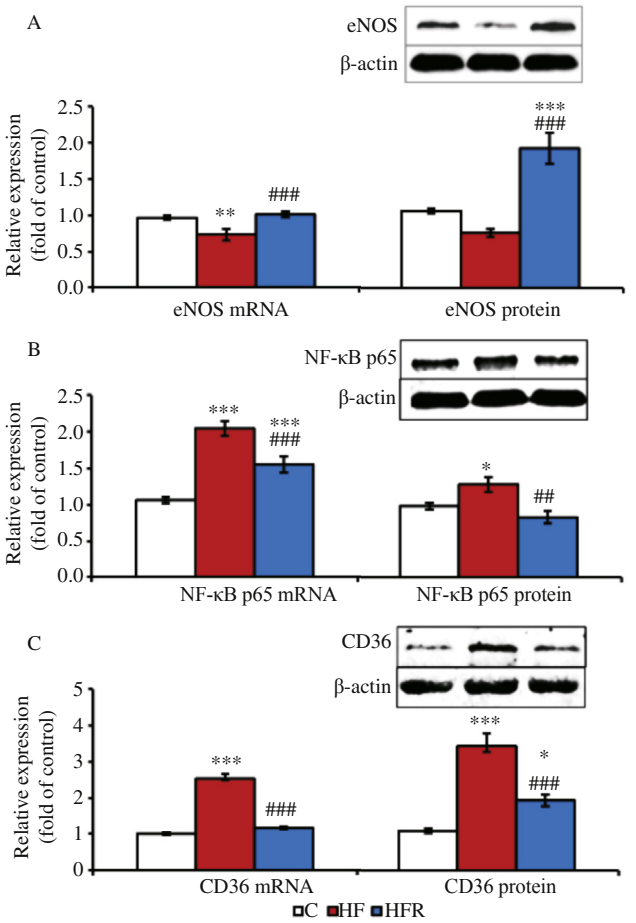

Figure 2. Aortic eNOS (A), NF-κB p65 (B), and CD36 (C) expression levels of the experimental groups.
Values are expressed as mean ± SEM ($n = 6$). *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ compared with Group I; ##: $P < 0.01$, ###: $P < 0.001$ compared with Group II. C: Group I; HF: Group II; HFR: Group III.

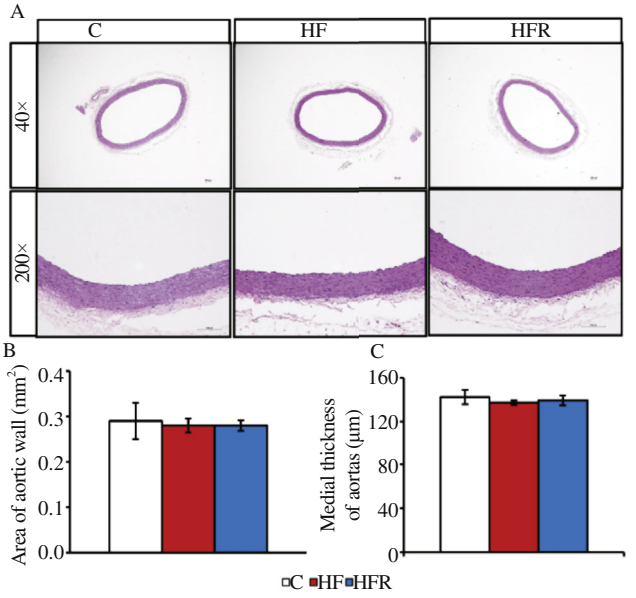

Figure 3. Aortic histology of the experimental groups.
A: H & E staining of representative aortic sections (upper panel, 40× magnification; lower panel, 200× magnification; scale bar = 100 μm); B: The area of the aortic wall; C: The tunica media thickness of the aortas. Values are expressed as mean ± SEM ($n = 3$). C: Group I; HF: Group II; HFR: Group III.

4. Discussion

The results of this study verified the effects of rice bran water extract from the Thai jasmine rice variety Khao Dawk Mali 105 on five major factors associated with the development of vascular disease in the metabolic syndrome, namely, visceral obesity, dyslipidemia, hyperglycemia, oxidative stress, and altered expression of eNOS, NF-κB p65, and CD36 in the aortic tissue.

Although the pathogenesis of the cardiovascular disease-related metabolic syndrome is not completely understood, high dietary fat intake is an important cause of cardiovascular risk factors in both human and experimental animals [23,24]. Therefore, the high-fat diet-fed rats were selected as a model for studying metabolic syndrome and related vascular risk factors. The present study is consistent with the previous data stating that the metabolic disturbances (increased body weight and intra-abdominal fat deposition, dyslipidemia, and hyperglycemia) were induced in the high-fat diet-fed male Sprague-Dawley rats as compared with the normal rats [25]. It is well established that these metabolic disturbances, including the increased circulating FFA levels, are involved in the development of cardiovascular disease [26,27]. Thus, the prevention of these metabolic disturbances is necessary for the reduction of cardiovascular disease. In the present study, the administration of rice bran water extract caused a significant decrease in the body weight and intra-abdominal fat deposition independently of food intake. Treatment with rice bran water extract also decreased total-C, TG, and FFA levels in serum and FBG without affecting the serum levels of LDL-C and HDL-C, which are similar to the previous observations [28]. Unlike our results, however, previous studies showed that the rice bran water extract treatment significantly increased the serum HDL-C, but decreased the serum LDL-C levels in animal models of metabolic syndrome [28,29]. These differences may be due to the types of rice bran, extraction methods, and experimental designs. In addition to metabolic disturbances, it is accepted that oxidative stress is associated with an increase in cardiovascular risk factors [2]. Hyperlipidemia and hyperglycemia are involved in the production of free radicals, leading to oxidant-antioxidant imbalance [30,31]. Previous studies have revealed that high-energy diet intake induced oxidative stress and lipid peroxidative damage in animal blood and aorta [3,30]. Consistent with these results, elevated concentrations of MDA were observed in both serum and aorta of rats fed with high-fat diet alone, thus indicating the existence of oxidative damage. Rice bran water extract treatment markedly decreased these oxidative damages in rats. These results are in agreement with those of Kang *et al.* showing that treatment with rice bran caused a significant decrease in plasma and erythrocyte MDA levels in high-fat diet-fed mice [32]. Justo *et al.* have also reported the antioxidant potential of rice bran enzymatic extract in obese rats by reducing the production of superoxide anion in their aorta [33]. Collectively, the current results indicated that rice bran water extract treatment had a tendency to decrease some of the risk factors associated with vascular diseases. Additional studies are needed to explore the mechanisms of how rice bran water extract treatment could reduce these vascular risk factors.

In the present study, the molecular mechanisms underlying the vasoprotective effects of rice bran water extract were clarified by analyzing the mRNA and protein expression levels of vascular genes involved in NO synthesis, inflammation, and lipid uptake. The hallmarks of vascular diseases associated with metabolic syndrome include the impairment of NO synthesis, chronic inflammation, and accumulation of lipid in the vascular wall. eNOS is a major enzyme that generates NO in the blood vessels as well as is an anti-inflammatory, anti-hypertensive, and anti-atherosclerotic molecule [5]. By contrast, NF-κB is considered as a proinflammatory transcription factor involved in the initiation of atherosclerosis [6]. In blood vessels, activation of NF-κB pathway in response to various atherogenic stimuli results in the production of proinflammatory molecules, such as tumor necrosis factor-α and inducible nitric oxide synthase. Moreover, CD36 is a scavenger receptor that regulates the lipid uptake and accumulation in monocytes, macrophages, and vascular cells [7]. Also, it is an important molecule in the formation of foam cells and atherosclerotic lesions. The results showed that eNOS was down-regulated while NF-κB p65 was up-regulated in the aorta of rats fed short-term high-fat diet. These findings are consistent with study of Wilson *et al.* reporting that the down-regulation of eNOS expression and up-regulation of NF-κB p65 expression were observed in the initial stage of vascular diseases [9]. Moreover, the finding that CD36 gene expression was increased in the aorta of Group II is similar to that of a previous study revealing significantly increased expression of CD36 in the aorta of high-fat-fed rats [10]. These observations suggest that the altered expression of eNOS, NF-κB p65, and CD36 in the metabolic syndrome could be the early signs of vascular diseases.

Interestingly, rice bran water extract treatment significantly increased eNOS mRNA and protein levels when compared with high-fat diet-fed rats, which is consistent with previous findings that the obese Zucker rats treated with the rice bran enzymatic extract showed higher eNOS protein levels in blood vessels [33]. Moreover, rice bran water extract treatment significantly inhibited high-fat diet-induced vascular NF-κB p65 expression in rats. The results are supported by previous studies reporting that supplementation with the rice bran enzymatic extract decreased the levels of proinflammatory markers (*e.g.*, tumor necrosis factor-α and inducible nitric oxide synthase) in the vasculature and serum of obese Zucker rats [29,33]. Wilson *et al.* reported that supplementation with rice bran decreased aortic fatty streak formation in hypercholesterolemic hamsters, suggesting that administration of rice bran may attenuate lipid uptake and deposition in the vascular wall [18]. Our finding that rice bran water extract treatment decreased CD36 expression in Group III compared with Group II clearly supports such a mechanism. Alterations in the expression of eNOS, NF-κB p65, and CD36 in the vascular cells and tissues have been shown to be associated with hypercholesterolemic, hyperglycemic, and pro-oxidant conditions [9,10,34–36]. Zhang *et al.* found that treatment with palmitate decreased eNOS expression in endothelial cells [37]. In addition, expression of NF-κB p65 has been reported to elevate in the endothelium of fatty acid-infused rats [38]. In the current study, the modulatory effects of rice bran water extract on the expression of these genes could be related to its hypocholesterolemic, hypoglycemic, FFA lowering, and antioxidant effects. Therefore, our study suggests that the regulation of eNOS, NF-κB p65, and CD36 expressions may contribute to the mechanisms by which rice bran water extract inhibits the molecular development of vascular diseases in a rat model of metabolic syndrome. Further experiments are required to explore the details of these mechanisms underlying the effects of rice bran water extract against vascular diseases. In this study, the effects of rice bran water extract on the aortic histology were also investigated. Unfortunately, the short-term treatment with the high-fat diet for

four weeks was not sufficient to induce the histologic abnormalities of the aortic wall in the rat model.

Many studies have suggested that the consumption of phenolic compounds and γ-oryzanols is associated with a decreased risk for metabolic syndrome and vascular diseases [39,40]. Our preliminary studies showed that rice bran water extract from the Khao Dawk Mali 105 rice variety contained low levels of these bioactive components. Based on previous evidence, the vasoprotective effects of rice bran water extract are probably due to the presence of other bioactive constituents such as protein and phytic acid, which have the anti-metabolic disorder and anti-atherogenic effects [32,41–43]. Furthermore, the report by Boonla *et al.* has indicated that treatment with peptides derived from the Khao Dawk Mali 105 rice bran significantly enhanced the endothelial function of two-kidney, one clip hypertensive rats by elevation of eNOS protein levels in the aorta and NO production [44]. In addition, treatment with these peptides significantly reduced the levels of plasma oxidative damage markers (MDA and protein carbonyl) and vascular superoxide anion production of rats. Evaluation of the bioactive compounds in rice bran water extract will be further performed to correlate their effects to the prevention of vascular disease.

Although rice bran water extract treatment did not reduce the overall risk factors for vascular disease, it was able to improve the expression of eNOS, NF-κB p65, and CD36 in the vasculature in high-fat diet-induced metabolic and oxidative stress. The present findings indicate that rice bran water extract is likely to prevent the initial development of vascular diseases by regulating cardiovascular risk factors as well as gene expression of eNOS, NF-κB, and CD36.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This work was supported by grants from the Research Unit, Faculty of Medicine, Thammasat University and the Higher Education Research Promotion and National Research University Project of Thailand (Grant number 2-17/2013 and 2014-76, respectively). We are very grateful to Dr. Nuntiya Somparn and Dr. Jarinyaporn Naowaboot (Department of Preclinical Science, Faculty of Medicine, Thammasat University, Thailand) for providing us with the MDA reagents and FFA assay kits, respectively.

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