Thai Rice Bran Protein Hydrolysate Causes Vasorelaxation in Isolated Mesenteric Vascular Beds of Insulin Resistance Rats

โปรตีนไฮโดรไลเสทจากรำข้าวไทยมีผลให้หลอดเลือดคลายตัวในหลอดเลือดมีเซนเทอริกที่แยกจากหนูแรทที่มีภาวะดื้ออินซูลิน

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ABSTRACT

The present study investigated the effect of Thai rice bran protein hydrolysate (RBP) on vascular responses in mesenteric vascular beds isolated from insulin resistance (IR) rats. Male Spraque-Dawley rats were fed with high-fat, high- carbohydrate (HFHC) diets and 15% fructose in drinking water for 16 weeks and mesenteric vascular beds were isolated. Results showed that rats fed with HFHC exhibited mild hypertension, an increase in fasting blood glucose (FBG), the impairment of oral glucose tolerance (OGTT), indicating insulin resistance. RBP produced vasorelaxation by dose dependent in the preparations with an intact endothelium. Removal of endothelium partially inhibited the vasorelaxant effect of RBP. In addition, capsaicin pretreatment with endothelial removal abolished the vasorelaxation response to RBP. Conclusion, RBP caused vasorelaxation mediating by vascular endothelium and TRPV1 receptors in mesenteric vascular beds of IR rats.

บทคัดย่อ

การศึกษาครั้งนี้เป็นการตรวจสอบผลของโปรตีนไฮโดรไลเสทจากรำข้าวไทยต่อการตอบสนองของหลอดเลือดมีเซนเทอริกที่แยกจากหนูแรทที่ทำให้เกิดภาวะดื้ออินซูลิน โดยให้หนู (Spraque-Dawley) เพศผู้ กินอาหารที่มีคาร์โบไฮเดรตและไขมันสูงและน้ำดื่มผสมน้ำตาลฟรุกโทส 15 เปอร์เซนต์ เป็นเวลา 16 สัปดาห์ ผลการทดลองพบว่าหนูมีความดันเลือดสูง ระดับน้ำตาลในเลือดสูง มีความบกพร่องของ oral glucose tolerance test ซึ่งบ่งชี้ภาวะดื้ออินซูลิน นอกจากนี้โปรตีนไฮโดรไลเสทจากรำข้าวมีผลให้หลอดเลือดคลายตัวตามความเข้มข้นในหลอดเลือดที่มีเซลล์ชั้นเอนโดทีเลียม และโปรตีนไฮโดรไลเสทจากรำข้าวทำให้การคลายตัวของหลอดเลือดลดน้อยลงในสภาวะที่ไม่มีเซลล์ชั้นเอนโดทีเลียม การให้ capsaicin ร่วมกับสภาวะที่ไม่มีเซลล์ชั้นเอนโดทีเลียมจะไม่พบการฤทธิ์การคลายตัวของหลอดเลือดต่อโปรตีนไฮโดรไลเสทจากรำข้าวเลย สรุปได้ว่าโปรตีนไฮโดรไลเสทจากรำข้าวมีผลให้หลอดเลือดคลายตัว โดยกลไกผ่านเซลล์ชั้นเอนโดทีเลียมและ TRPV1 receptors ของหลอดเลือดมีเซนเทอริกแยกจากหนูทดลองที่ถูกเหนี่ยวนำให้เกิดภาวะดื้ออินซูลิน

Key Words: Thai rice bran protein hydrolysate, Vasorelaxation, Insulin resistance
คำสำคัญ: รำข้าวไทย การคลายตัวของหลอดเลือด ภาวะดื้ออินซูลิน

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Introduction

The increase in consumption of high fat and high carbohydrate (HFHC) diets and fructose is now accepted that it is able to contribute metabolic syndrome including glucose intolerance, insulin resistance, central obesity, dyslipidaemia, and hypertension (Basciano *et al.*, 2005; Eckel *et al.*, 2005; Hunt *et al.*, 2004; Lakka *et al.*, 2002). Previous study, we have investigated the high-fat, high-fructose diet-induced an insulin resistance rat model including high insulin level, high fasting blood glucose, impaired glucose tolerance and high blood pressure (Bunbupha S *et al.*, 2012). It is well established that animals with insulin resistance are related to endothelial dysfunction and cardiovascular disease (Chen *et al.*, 2012). Endothelium is the major regulator of vascular homeostasis, especially maintenance of vascular tone by releasing vasodilator substances such as nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factor (EDHF)) and vasoconstrictor substances including endothelin and angiotensin II (Davignon and Ganz, 2004). Moreover, recent study has been reported that transient receptor potential cation channel subfamily V member 1 or TRPV1 receptor mainly located on perivascular nerves and vascular endothelium that mediated vasorelaxation. Capsaicin stimulates TRPV1 receptor on perivascular nerves causes the release of calcitonin gene-related peptide (CGRP) that acts on vascular receptors and activates vasorelaxation (Kawasaki *et al.*, 2009). On vascular endothelium, TRPV1 receptors was responded to a vasoactive agent to induce the release of CGRP or NO (Poblete *et al.*, 2005)

Rice bran is the pericarp and germ of *Oryza sativa* seeds. Several studies reported the nutritional quality of rice bran, which is composed of oil, protein, fiber, vitamins, and minerals. The beneficial effects of RBP have been demonstrated such as antihypertensive, immunomodulatory, antimicrobial, antioxidant, antithrombotic and hypocholesterolemic effect. In addition, RBP strongly showed ACE inhibitory activity in vitro (Li *et al.*, 2007). Previous study reported that RBP cause vasorelaxation in both conduit artery (thoracic aorta) and resistance artery (mesenteric vascular beds) in hypertensive rats (Tuangpolkrung *et al.*, 2011). Since the mesenteric circulation, which is the largest vascular bed, influences regulation of systemic blood pressure (Jin *et al.*, 2010). However, little information is known regarding the effect of rice protein hydrolysates on vascular responses in the vascular bed of rats with insulin resistance. The present study investigated whether RBP produces vasorelaxation in mesenteric vascular beds of insulin resistance rats induced by HFHC diets.

Objectives

1. To investigate the vasorelaxation effect of Thai rice bran protein hydrolysate on resistance artery (mesenteric vascular beds) of rats with insulin resistance.
2. To investigate the mechanism of Thai rice bran protein hydrolysate mediates vasorelaxation in mesenteric vascular beds of rats with insulin resistance.

Materials and methods

Preparation of RBP

RBP were extracted from defatted Hom-Mali rice bran under alkali condition (pH 11) followed by acid precipitation at isoelectric point (pH

4.5) (Jiamyangyuen *et al.*, 2005). The crude extract contained 56.31% protein (dry basis). RBP were prepared from the suspension of rice bran protein extract (RBPE: water = 1:7). Enzymatic hydrolysis was performed at 3% of a bacterial alkaline protease, Protease G6 (enzyme to protein ratio = 3:97), pH 8.0, 55°C for 4 hours (Timachai and Thawornchinsombut, 2011). The hydrolysis was inactivated at 85°C for 15 min, cooled, and centrifuged at 10,000 g for 10 min at 4°C. The supernatant was filtered through ultrafiltration membrane with the 50 kDa MW cut-off). The RBP MW < 50 kDa was freeze dried, kept in a tight, light-protected container and stored at -20 °C until use. Yield of RBP (50 kDa) was about 6.5%-7.5% which was calculated based on defatted rice bran. RBP was dissolved in distilled water before used (Timachai and Thawornchinsombut, 2011).

Animals

Adult male Sprague-Dawley rats weighing 200 g were purchased from National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom, Thailand. All animals were housed in the HVAC (Heating, Ventilation and Air-Conditioning) System with 12 hours dark/light cycle at the Northeast Laboratory Animal Center, Khon Kaen University, Thailand. All animal procedures were controlled and approved by the Institutional Animal Ethics Committee of Khon Kaen University (AEKKU 19/2555).

Experimental design and metabolic syndrome induction

After one week of acclimatization, the animals were fed with high fructose, high fat and high carbohydrate diet for 16 weeks to induce insulin resistance conditions. The compositions of diet were fructose, lard, sweetened condensed milk, distilled water, standard chow and salt mix and 15% fructose in drinking water. During 16 week, indirect blood pressure (BP), fasting blood glucose (FBG), and oral glucose tolerance (OGTT) were measured once a month. After 16 weeks of insulin resistance induction, rats were anesthetized with pentobarbital sodium 60 mg/kg body weight, after that mesenteric vascular beds were isolated as described previously (Pakdeechote *et al.*, 2007) and vasorelaxation mechanisms of RBP were explored in intact and absent endothelium conditions. In brief, mesenteric vascular beds were immediately removed. The abdominal cavity was open and the main branch of the superior mesenteric artery was identified, cleaned of connective tissues and annulated with a blunted hypodermic needle (No.21). The superior mesenteric vein was cut and the mesenteric vascular bed was flushed gently with Krebs' solution (0.5 ml). Subsequently, the mesenteric vascular bed was separated from gut by carefully cutting close to the intestinal wall. The mesenteric vascular bed preparations was placed on a stainless steel grid in a warm humid chamber (37°C) and perfused at a constant flow rate of 5 ml/min, using a peristaltic pump (Cole-Palmer Instrument, Illinoi, U.S.A.). Krebs' solution was composed of the following (mM): NaCl 118.2, KCl 4.7, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 1.18, Glucose 11.0, $NaHCO_3$ 25 and $CaCl_2.2H_2O$ 1.25 (pH 7.4). The solution was maintained at 37°C and continually gassed with 95% O_2 and 5% CO_2. An air removal system was incorporated to prevent denudation of the endothelium by air entrapment in the perfusate. Mesenteric vascular responses were detected as changes in perfusion pressure (mmHg). Mean perfusion pressure was monitored using a pressure

transducer and the data were recorded using the BIOPAC system (Santabara Inc., California, U.S.A.). To remove the vascular endothelium, preparations were perfused with sodium deoxycholate (1.8 mg/ml in saline) for 30 s. This cause a transient increase in perfusion pressure (20-30 mmHg). The preparation was then rinsed with Kreb's solution for 30 min to wash out sodium deoxycholate (Shiraki *et al.*, 2000). Then, preparations were pretreated with capsaicin, followed by a 15 minute washout period to deplete a CGRP. After an initial 30 min equilibration period, methoxamine was added (5 uM) in order to raise tone. RBP was added into the perfused vascular bed in a cumulative dose manner ranging from 0.001-1 mg/ml.

Results

Effect of HFHC diets on rats' body weight, FBG, OGTT and systolic blood pressure (SBP)

Rat received HCHF diets showed a body weight gain from 258.12 ± 1.85 g (0 week) to 398.71 ± 7.52 g (16 weeks). Fasting blood glucose (at week 0, 4, 8, 12, 16) was gradually increased in rat fed with HCHF diets as shown in Figure 1. Furthermore, the area under the curve (AUC) of OGTT was significantly increased in week 12 and 16 when compared to those of the baseline (week 0) (Figure 2) indicating an insulin resistance conditions in rats received HFHC diets. At 16 weeks of HFHC feeding, the increase of SBP was observed (141.3 ± 1.81 mmHg) compared to the value before HCHF diet feeding (124.48 ± 3.58 mmHg) (Figure 3).

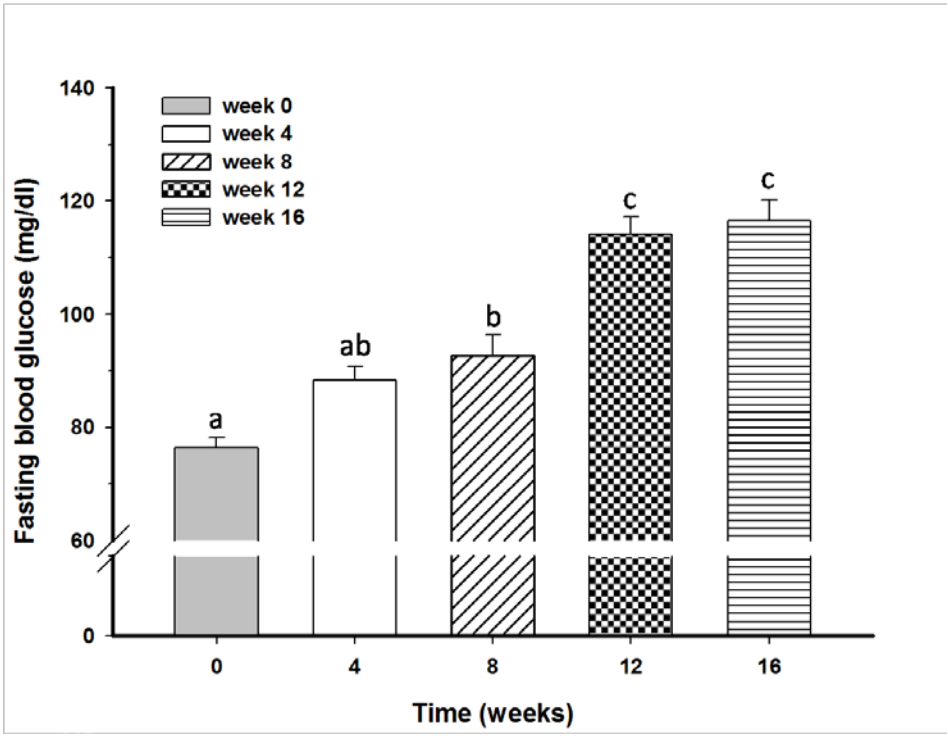

Figure 1 Effect of HFHC diets on fasting blood glucose during 16 weeks of study period, letters above the columns indicate statistical significance, with dissimilar letters meaning significance at $p < 0.05$ and the same letters meaning no significance

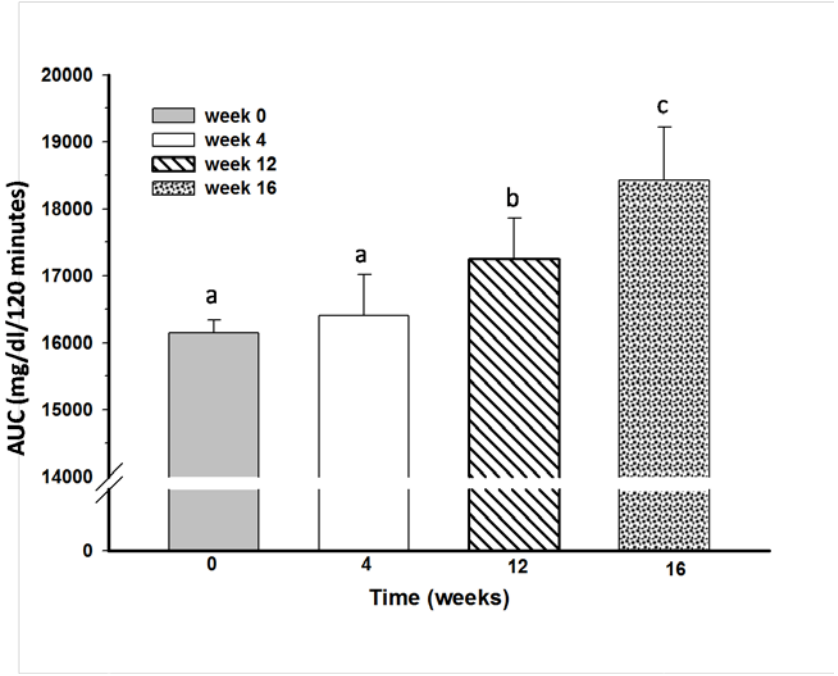

Figure 2 Effect of HFHC diets on OGTT expressed as area under the curve (AUC) during 16 weeks of the study period, letters above the columns indicate statistical significance, with dissimilar letters meaning significance at $p < 0.05$ and the same letters meaning no significance

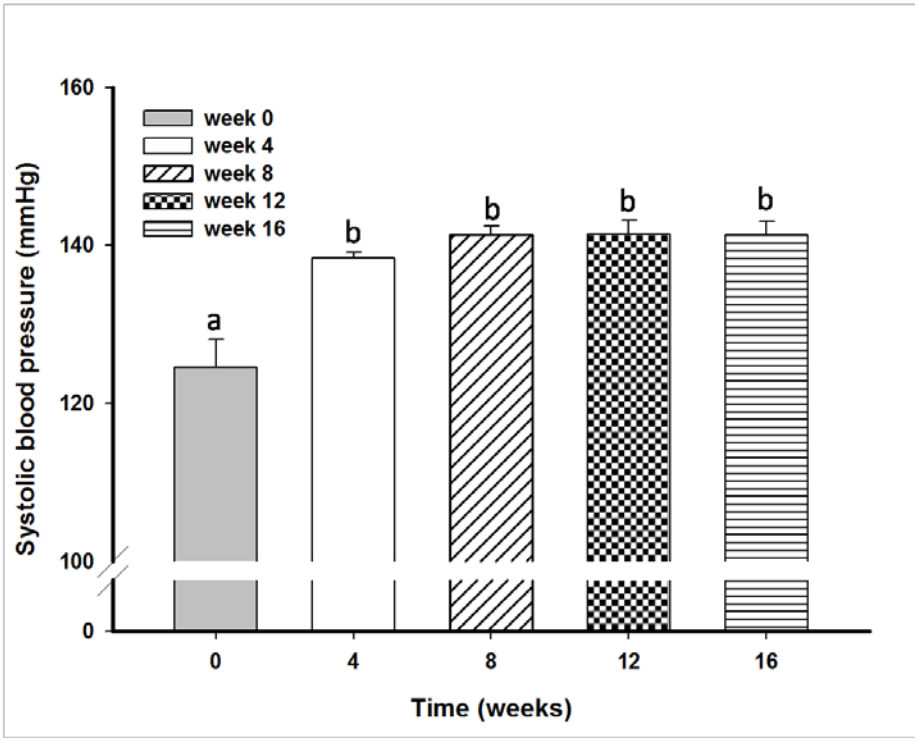

Figure 3 Effect of HFHC diets on SBP (mmHg) during 16 weeks of study period, letters above the columns indicate statistical significance, with dissimilar letters meaning significance at $p < 0.05$ and the same letters meaning no significance

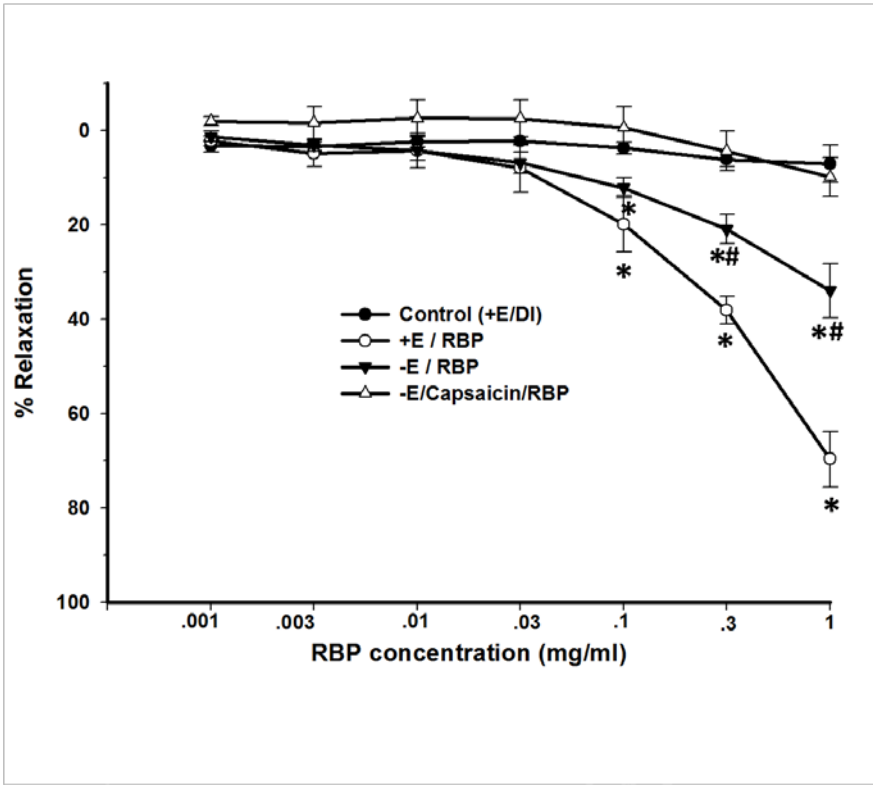

Figure 4 Concentration-response curves of RBP induced vasorelaxation in mesenteric vascular beds. +E represents preparations with an intact endothelium, - E represents preparations with denuded endothelium, -E/Capsaicin represents preparations with denuded endothelium and after capsaicin pretreatment. Data are presented as mean ± S.E.M. # $P < 0.05$, compared with +E/RBP, *$P < 0.05$, compared with control (+E/DI) in the same volume.

Vasodilator responses to RBP under methoxamine raised tone conditions

RBP (0.001- 1 mg/ml) produced a concentration-dependent relaxation of mesenteric vascular beds with an intact endothelium. While, there was no response to de-ionized water (DI) in the preparations with intact endothelium (control). The vasorelaxation effect of RBP in preparations with a denude endothelium was significantly attenuated. Interestingly, capsaicin pretreatment in mesenteric beds with a denuded endothelium abolished RBP induced vasorelaxation (Figure 4). The result indicated that the relaxant effect of RBP requires the endothelium and TRPV1 receptor.

Discussion

The present study has shown that HFHC diets induced IR in rats as well as hypertension. This was supported by the increases in SBP, FBG, impaired glucose tolerance in rats received HFHC diets. The result also provides the first evidence that RBP induced vasorelaxation in resistance vascular beds isolated from IR rats.

In the animal model of IR rats, it has well established that increases in consumption of high-fat, high carbohydrate diets had the adverse effects such as vascular dysfunction, hypertension, dyslipidemia, insulin resistance and obesity (Ble-Castillo *et al.*, 2012). Panchal and coworkers (2012) reported the

increase in blood pressure in this animal model which was primarily due to the increased production of reactive oxygen species and impaired endothelial function (Panchal *et al.*, 2012). Our results were consistent with above that HFHC diets caused insulin resistance in rats. Additionally, RBP exhibited vasodilator effects in the rat mesenteric vascular bed with an intact endothelium of IR rats under methoxamine raised tone conditions. Removal of endothelium partially inhibited the vasorelaxation response to RBP. Moreover, capsaicin pretreatment, to deplete the CGRP (Pakdeechote *et al.*, 2007), and endothelium removal abolished the vasodilator effects of RBP. The result indicating that the relaxant effect of RBP requires the endothelium and TRPV1 receptors. TRPV1 receptor is known as the capsaicin receptor. Activation of TRPV1 has been shown to exert antihypertensive effect through stimulation the release of neurotransmitter, calcitonin gene-related peptide (CGRP) or nitric oxide which is a potent vasodilator (Jin *et al.*, 2012; Poblete *et al.*, 2005). Previous study suggested that RBP induced vasorelaxation in mesenteric vascular bed plausible mechanism by endothelium dependent vasodilation and endothelium independent vasodilation which TRPV1 receptor was involved (Tuangpolkrung *et al.*, 2011).

Conclusion

Results of this study demonstrated that HFHC diet together with high fructose in water induces IR in rats. RBP induced vasorelaxation in mesenteric vascular beds of IR rats. The relaxant effect of RBP was mediated primarily via endothelium pathway. In addition to endothelium involvement, vasorelaxation induced by RBP was also partially attributed by TRPV1 receptor located at perivascular nerves.

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