Resistant starch isolated from Luem-Pua glutinous rice decreases adipocyte size of visceral fat and thickness of thoracic aorta in high-fat diet-fed rats

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Background : *Luem-Pua glutinous rice has been reported to contain a high content of resistant starch (RS). Previous studies showed weight loss, decreased blood glucose and lipid profiles levels, as well as decreased hyperplasia and hypertrophy of adipocytes in RS treatment. However, the study of protective effects of RS isolated from Luem-Pua glutinous rice on obesity is still needed to provide supportive information for potential use as dietary supplements or development of therapeutic agents for the treatment obesity.*

Objective : *We aimed to investigate the effects of RS isolated from Luem-Pua glutinous rice on body weight and visceral fat weight, blood parameters, histological changes of visceral fat and thoracic aorta in high-fat diet-fed rats.*

Methods : *Eighteen male rats were divided into three groups (n = 6/group) and daily treated as follows: control, normal diet; HF+DW, a high-fat diet with distilled water; HF+LP, a high-fat diet with RS isolated from Luem-Pua glutinous rice. We investigated food intake (FI), final body weight (FBW) and length (FBL), serum glucose, serum lipid profiles. In addition, histology of visceral fat and thoracic aorta were investigated by hematoxylin and eosin (H&E) staining at the end of the experiment.*

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Results : *FI and FBW showed significantly negative correlation in the HF group, especially in HF+LP. FBW and visceral fat weight of HF+LP and HF+DW were significantly increased in comparison to control group. FBL and all blood profiles were not significantly different among the three groups. Ratio of adipocyte area to number and thickness of the thoracic aorta were significantly reduced in HF+LP in comparison to control and HF+DW groups*

Conclusion : *This study addressed specific protective effects of RS isolated from Luem Pua glutinous rice on obesity as follows: 1) reduction of adipocytes size, 2) prevention of pathological changes of thoracic aorta, and 3) decreasing of thoracic aorta thickness in high-fat diet-fed rats.*

Keywords : *Luem-Pua glutinous rice, resistant starch, visceral fat, thoracic aorta, high-fat diet.*

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เหตุผลของการทำวิจัย : *ข้าวเหนียวสายพันธุ์ลืมผัวเป็นข้าวเหนียวที่ถูกพบว่ามีปริมาณของแป้งทนย่อยเป็นจำนวนมาก ซึ่งจากการวิจัยพบว่าแป้งทนย่อยสามารถลดน้ำหนักตัว ลดขนาดและจำนวนของเซลล์ไขมัน ลดระดับน้ำตาลและไขมันในเลือด อย่างไรก็ตามการศึกษาประโยชน์ของแป้งทนย่อยที่สกัดจากข้าวเหนียวสายพันธุ์ลืมผัวยังขาดข้อมูลสนับสนุนอยู่มาก ผู้วิจัยจึงเล็งเห็นความสำคัญในการศึกษาครั้งนี้เพื่อหวังว่าจะนำข้อมูลที่ได้มาพัฒนาข้าวเหนียวสายพันธุ์ลืมผัวให้เป็นอาหารเสริมหรือยาที่ใช้ในการป้องกันหรือรักษาโรคอ้วนได้ในอนาคต*

วัตถุประสงค์ : *เพื่อศึกษาประโยชน์ของแป้งทนย่อยที่สกัดจากข้าวเหนียวสายพันธุ์ลืมผัวต่อการเปลี่ยนแปลงของน้ำหนักตัว ระดับน้ำตาลและไขมันในกระแสเลือด ลักษณะทางจุลกายวิภาคของไขมันในช่องท้องและหลอดเลือดแดงเอออร์ตาในช่องอกในหนูที่เหนี่ยวนำด้วยอาหารผสมไขมันสูง*

วิธีการทำวิจัย : *ทำการศึกษาในหนูทดลองจำนวน 18 ตัว โดยแบ่งกลุ่มการทดลองเป็น 3 กลุ่ม กลุ่มละ 6 ตัว ดังนี้ กลุ่มควบคุมจะได้รับอาหารตามปกติ กลุ่มทดลองที่ได้รับอาหารปกติที่ผสมไขมันสูงร่วมกับให้สารสกัดแป้งทนย่อย และกลุ่มทดลองที่ได้รับอาหารปกติที่ผสมไขมันสูงร่วมกับให้สารสกัดหลอก โดยจะทำการวัดปริมาณของอาหารที่กิน น้ำหนักและความยาวตัวหนู วัดค่าระดับน้ำตาลและไขมันในกระแสเลือด และเมื่อสิ้นสุดสัปดาห์ที่ 18 ทำการการุณยฆาตหนู ชั่งน้ำหนักตัว น้ำหนักอวัยวะและเก็บไขมันในช่องท้องและหลอดเลือดแดงเอออร์ตาในช่องอกเพื่อนำมาศึกษาลักษณะทางจุลกายวิภาคต่อไป*

ผลการศึกษา : *ผลการศึกษาพบว่าปริมาณของอาหารที่กินในหนูกลุ่มทดลองที่ได้รับอาหารปกติที่ผสมไขมันสูงทั้งสองกลุ่มจะแปรผกผันกับน้ำหนักตัว ค่าน้ำหนักตัวและน้ำหนักไขมันในหนูกลุ่มทดลองที่ได้รับอาหารปกติที่ผสมไขมันสูงทั้งสองกลุ่มพบว่ามีค่ามากกว่ากลุ่มควบคุม แต่ค่าความยาวตัวหนูและระดับน้ำตาลและระดับไขมันในกระแสเลือดพบว่าไม่มีความแตกต่างกันเมื่อเปรียบเทียบทั้งสามกลุ่ม เมื่อทำการศึกษาลักษณะทางจุลกายวิภาคของเซลล์ไขมันในช่องท้องและหลอดเลือดแดงเอออร์ตาในช่องอกพบว่า ในหนูกลุ่มทดลองที่ได้รับสารสกัดแป้งทนย่อยจะมีขนาดขนาดของเซลล์ไขมันในช่องท้องและความหนาของหลอดเลือดแดงเอออร์ตาในช่องอกน้อยกว่ากลุ่มทดลองที่ได้รับสารสกัดหลอก*

สรุป : *ในการศึกษาครั้งนี้ พบว่าประโยชน์ของแป้งทนย่อยที่สกัดจากข้าวเหนียวสายพันธ์ลืมผัวมีผลช่วยลดขนาดของเซลล์ไขมันในช่องท้อง ป้องกันการเกิดพยาธิสภาพของหลอดเลือดแดงเอออร์ตาในช่องอก และลดความหนาของหลอดเลือดแดงเอออร์ตาในช่องอกในหนูที่เหนี่ยวนำด้วยอาหารที่ผสมไขมันสูง*

คำสำคัญ : *ข้าวเหนียวสายพันธ์ลืมผัว, แป้งทนย่อย, ไขมันในช่องท้อง, หลอดเลือดแดงเอออร์ตาในช่องอก, อาหารที่ผสมไขมันสูง.*

The prevalence of obesity is increasing due to a westernized lifestyle and consumption of fast food. In 2015, the World Health Organization reported that approximately 1.6 billion adults worldwide were overweight and more than 400 million were obese. (1-3) A high-fat diet is a major risk of obesity due to an increasing energy intake, disturbed energy balance and a resulting increased risk of cardiovascular disease or cancer. (4, 5) The percentage of high-fat food used in several animal studies was between 30% - 60% of daily standard food over about four weeks. It has been revealed that such percentages of high-fat diet from many fat types, for example lard, sunflower oil, fish oil, and coconut fat, can promote metabolic disorders. (6) Luem-Pua glutinous rice, pigmented Thai rice, has been reported to contain phenolics, flavonoid, α-tocopheral, and resistant starch (RS). (7, 8) RS cannot be digested by enzymes in the small intestine, and it is fermented in the colon by microbes. There are four types of RS: RS1, RS2, RS3, and RS4 based on digestive capability.(9, 10) Feeding with food and oral gavage with 16% of RS could reduce total energy intake, weight gain and fat pad mass.(11) Another study has reported that treatment with food containing 55% RS in obese rats showed a reduction in mesenteric adipose tissue weight and an increase of the number of small adipocytes.(12) In 2004, Park et al. has reported that RS consumption reduced the levels of serum glucose, total cholesterol, and low-density lipoprotein cholesterol (LDL-cholesterol).(13) Additionally, Bronkowska et al. showed that serum triglyceride was decreased in rats receiving the oil- or lard-containing diets supplemented with 10% of RS4 in comparison to the control group. (14) However, the effects of RS isolated from Luem-Pua glutinous rice on obesity needed to be assessed, to provide the supportive information for potential use as dietary supplements or development of therapeutic agents for obesity. In this study, we therefore investigated effects of RS isolated from Luem-Pua glutinous rice on the body and visceral fat weights, blood parameters including serum glucose, total cholesterol, triglyceride, and HDL-cholesterol, and histological changes of visceral fat and thoracic aorta in the high-fat diet-fed rats.

Materials and Methods

Preparation of rice resistance starch

One hundred g of Luem Pua glutinous rice sample was soaked with 500 ml of distilled water in ratio of 1:5 (w/v) for 2 hours and then put into an automatic rice cooker about 30 min. Then, the volume was adjusted to 2 L and homogenized at medium speed of the blender for 5 min. The rice paste sample was passed through heat-moisture treatment (HMT) by autoclaving at 121 ^{o}C for 1 h and cooled at room temperature for 2 h. The sample was kept at 4 ^{o}C for 24 hours before frozen and thaw at room temperature for 1 h. The sample was then re-autoclaved and kept at 4 ^{o}C for 24 h. Finally, frozen rice paste sample was freeze-dried. The rice powder sample obtained from freeze-drying was ground in an electric coffee grinder and kept in polyethylene bags which stored at -20 ^{o}C until used.

Animals and diets

All animal experiments have been approved by the Ethics Committee on Animals Experiments of the School of Medicine, Mae Fah Luang University.

Eighteen adult male Wistar rats (230 - 250 gram) were purchased from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand. The rats were housed in individual cages having free access to food and water. They were kept under a 12:12 h light: dark cycle at a controlled temperature (24–26 ^{o}C). The rats were habituated for 1 week before investigation. The animals were divided randomly into three groups of six: control group was fed with standard food for 18 weeks; high-fat diet with distilled water (HF + DW) was fed with a mix of 50% of saturated fat (lard) in standard food for 10 weeks, and then they were continually fed with the same mixed food and were also given gavage with distilled water at a volume of 4 ml/kg body weight per day for 8 weeks; and, high-fat diet with RS isolated from Luem Pua glutinous rice (HF + LP) was fed with the same high fat mixed food, but was also given by oral root gavage with the RS isolated from Luem Pua glutinous rice at a dosage of 2 g/kg body weight per day for 8 weeks.

Biological analysis

Food intake (FI), final body weight (FBW) and length (FBL) were measured every week. The blood samples were collected from a tail vein at commencement and then every two weeks. Blood samples were analyzed to determine serum glucose and serum lipid profiles. At the end of the experiment, each rat was euthanized by intra-peritoneal injection of pentobarbital sodium (150 mg/kg, i.p.). The rats were dissected their internal organs: thoracic aorta, liver, kidneys, small intestine with mesentery were removed. The visceral fat including epididymal and retroperitoneal fat were also removed and weighed. Samples, including thoracic aorta, epididymal, and retroperitoneal fats, were washed in PBS and immediately fixed in 10% formalin for 2 weeks until just before the analysis.

Histological study

Tissue samples were processed using an automatic tissue-processing machine (Citadel 1000, Shandom Company). The tissue processing method consisted of three steps; dehydration, clearing and infiltration. Briefly, the tissue samples were dehydrated with a graded series of ethanols, xylene, and infiltrated in paraffin. Then, the tissues were embedded in paraffin by using the embedding machine (Kunz instrument WD-4, Shandon Company). The sample paraffin blocks were sectioned at a thickness of 5 μm by the semiautomatic microtome (Thermo Sciencetific Microm HM 325, Germany) and the sections were mounted on glass slides. Once dry, the slides were stained with Harris' hematoxylin and eosin (H&E) using standard protocol. After that, the sections were mounted with Permount (Fisher Scientific) and cover-slipped.

Image analysis

H&E staining of adipose tissues and thoracic aorta were investigated under the Olympus B $\times$ 51 light microscope (Olympus optical CO., LTD., Japan). Histological images were randomly captured along the longitudinal line of tissues at 4 $\times$, 10 $\times$, and 20 $\times$ objectives using an Axiocam 105 color digital camera (Carl Zeiss microscopy, Germany). Three different areas per rat of each organ were analyzed using an Image J analysis. To measure number and area of adipocytes, we determined using specific criteria: (1) all three pictures were captured at low power field

(40 × magnification); (2) the adipocytes were identified by the cell membrane and also showed an outline of the cell profiles; (3) the adipocytes were sphere-like shape and had the area between 1,000 and 30,000 μm^2; and (4) the adipocytes bordering the picture frame were excluded. To measure thoracic aorta thickness, all three pictures were captured at low power field (200 × magnification); (2) the thoracic wall showed intact in *tunica intima, tunica media*, and *tunica adventitia*. The NIS elements software was used to capture and measure the thickness of thoracic aorta.

Statistical analysis

The results are represented as mean ± standard error mean (S.E.M.). The means were compared using one - way analysis of-variance (ANOVA). The strength of correlation between the two variables was determined by Pearson's Sample Correlation Coefficient. All of the statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software version 17.0 and MegaStat (version 10.4) software. Statistical significance was defined as $P < 0.05$.

Results

Strongly negative correlation between food intake and body weight in HF + LP during 18 weeks

Mean of food intake in the control group was higher than that in the HF + DW and the HF + LP groups after week 5 of the experiment, but the mean of body weight of the control group was lower than those in the other groups throughout all weeks of the experiment (Figure. 1A). The correlation between the food intake and body weight of each group is shown in Figure.1C-E. The HF + LP group showed significantly negative correlation between the food intake and body weight (r = - 0.73, P = 0.02).

Increasing of final body weight and fat weight in high-fat diet-fed rats

Table 1 shows the parameters including FBW, FBL, body mass index (BMI), organ weight and visceral fat weight of rats compared among three groups. The FBW, epididymal fat weight, and retroperitoneal fat weight were significantly increased in HF + DW and HF + LP in comparison to control group. However, the small intestine with mesentery, epididymal fat, and retroperitoneal fat weights in the HF + LP were lower than those in the HF + DW, but it did not reach the significant level.

High triglycerides, total cholesterol/HDL ratio, and triglycerides/HDL ratio in HF + LP fed rats

Serum glucose, total cholesterol, triglycerides, and HDL-cholesterol ratios of rats at 18th week were divided by 1st week and compared among the three groups. In addition, total cholesterol/HDL and triglycerides/HDL ratios at 18th week were compared among three groups. The results showed that ratio of total cholesterol in high-fat diet-fed rats were higher than that in the control group. However, the ratio of triglyceride, total cholesterol/HDL, and triglyceride/HDL in HF + LP were significantly higher than that of the control and HF + DW groups (Table 2).

Decreasing of adipocyte area/number ratio of visceral fat in HF + LP fed rats

The result shows that adipocytes in the visceral fat of the HF + DW were larger than in the control group. Moreover, adipocytes size of the HF +

LP was decreased when compared with the HF + DW group (Figure. 2A). Mean of adipocyte number, area, and ratio of adipocyte area/number of retroperitoneal fat and epididymal fat are shown in Figure. 2B,C. Mean of adipocyte number of visceral fat in HF + LP were lower than that in the control and HF + DW group. Mean of adipocyte area and ratio of adipocyte area/number in visceral fat of HF + LP were significantly higher than that in control and HF + DW.

Preventing of aberrant histology and decreasing of thoracic thickening in HF + LP fed rats

Histology of the thoracic aorta was observed and compared among the control, HF + DW, and HF + LP groups. Histological changes of the thoracic aorta, including pale cytoplasm of cells, huge cytoplasmic vacuoles infiltration, irregular arrangement of smooth muscle cells in *tunica media*, and closely packed of adipose tissue in the *tunica adventitia*, were prominently found in the HF + DW group. In the HF + LP group, the smooth muscle cells in *tunica media* were more regularly arranged and the amounts of cytoplasmic vacuoles were rare (Figure. 3A). Moreover, the thoracic aorta thickening of HF + LP was significantly lower than that in the HF + DW group (Figure. 3B).

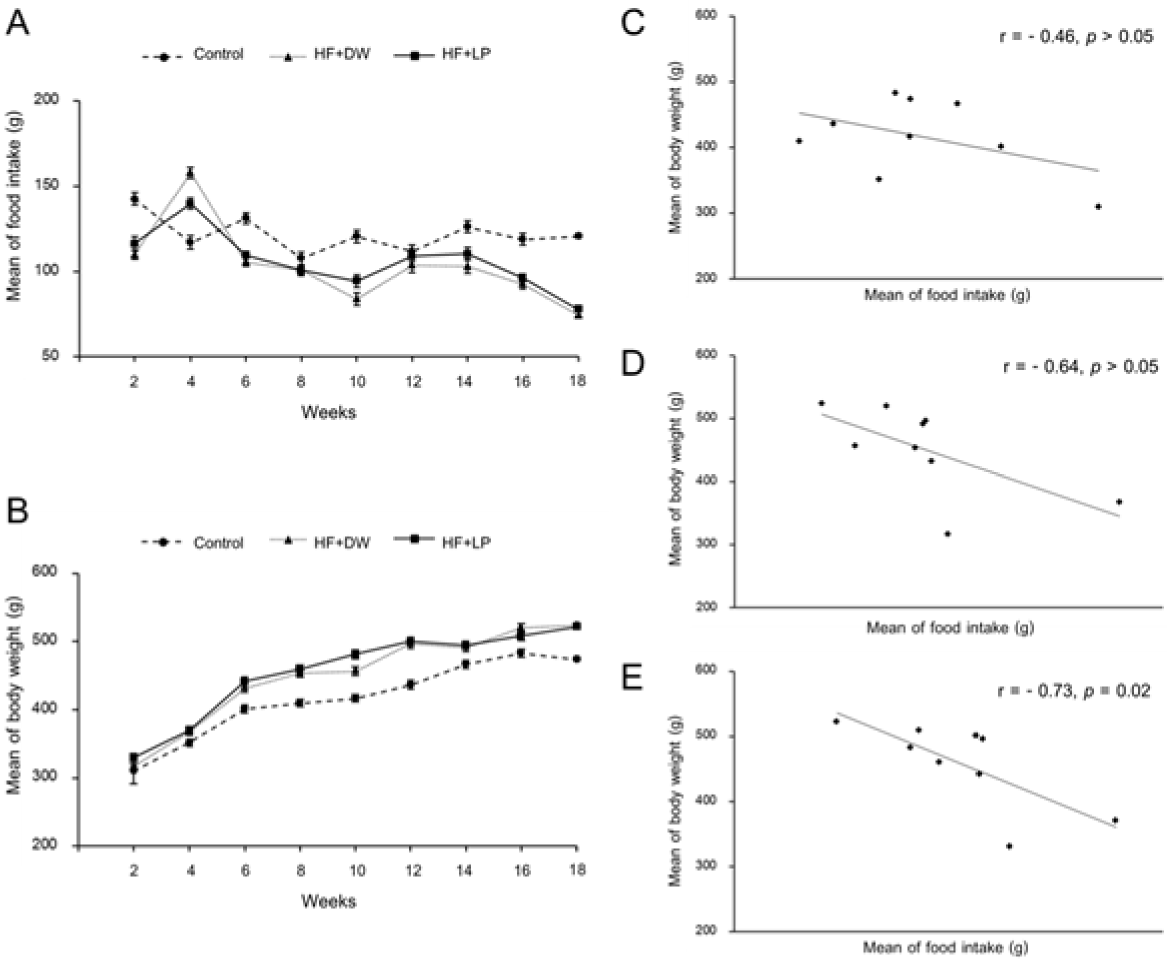

Figure 1. (A) Mean of food intake and (B) mean of body weight compared among different three groups: control, HF + DW: a high-fat diet with distilled water, and HF + LP: high-fat diet with Luem Pua. (C-D) The correlation between food intake and body weight in control, HF + DW, and HF + LP group were determined using Pearson's correlation, $P < 0.05$. Values are presented as mean ± S.E.M.

Table 1. Final body weight (FBW), final body length (FBL), body mass index (BMI), organs weight and visceral fats weight of rats compared among the three groups.

Parameters	**Control**	**HF+DW**	**HF+LP**
Final body weight (g)	475.00 ± 14.78	528.33 ± 15.18*	525.00 ± 15.22*
Final body length (cm)	24.08 ± 0.24	24.50 ± 0.52	24.67 ± 0.33
BMI (g/cm^2)	0.81 ± 0.01	0.88 ± 0.03	0.86 ± 0.01
Liver (g)	12.94 ± 0.27	11.62 ± 0.81	11.36 ± 0.73
Kidney and adrenal gland (g)	2.21 ± 0.05	2.26 ± 0.03	2.22 ± 0.06
Small intestine with mesentery (g)	16.87 ± 0.43	19.48 ± 1.62	17.04 ± 0.94
Epididymal fat weight (g)	13.30 ± 1.56	19.29 ± 1.74*	18.74 ± 1.13*
Retroperitoneal fat weight (g)	14.34 ± 1.58	22.97 ± 3.0*	21.07 ± 1.53*

Data are presented as mean ± S.E.M., *n* = 6 per group

* Significant at *P* < 0.05 level compared with control group

Table 2. The Wk 18/Wk 1 serum glucose, total cholesterol, triglyceride, and HDL-cholesterol ratios of rats compared among the three groups. Wk 18 total cholesterol/HDL and triglyceride/HDL ratios of rats compared among the three groups.

Ratios of blood parameters	**Control**	**HF+DW**	**HF+LP**
Wk 18/Wk 1 serum glucose	1.38 ± 0.09	1.32 ± 0.08	1.21 ± 0.06
Wk 18/Wk 1 total cholesterol	1.04 ± 0.06	1.12 ± 0.04	1.21 ± 0.03
Wk 18/Wk 1 triglyceride	1.26 ± 0.08	1.25 ± 0.13	2.03 ± 0.02*,□
Wk 18/Wk 1 HDL-cholesterol	1.19 ± 0.05	1.07 ± 0.04	1.09 ± 0.27
Wk 18 total cholesterol/HDL	1.06 ± 0.01	1.14 ±0.03	1.22 ± 0.05*
Wk 18 triglyceride/HDL	0.91 ± 0.10	0.92 ± 0.06	1.39 ± 0.09*,□

Data are presented as mean ± S.E.M., *n* = 6 per group

*,□ Significant at *P* < 0.05 level compared with control group and HF ± DW

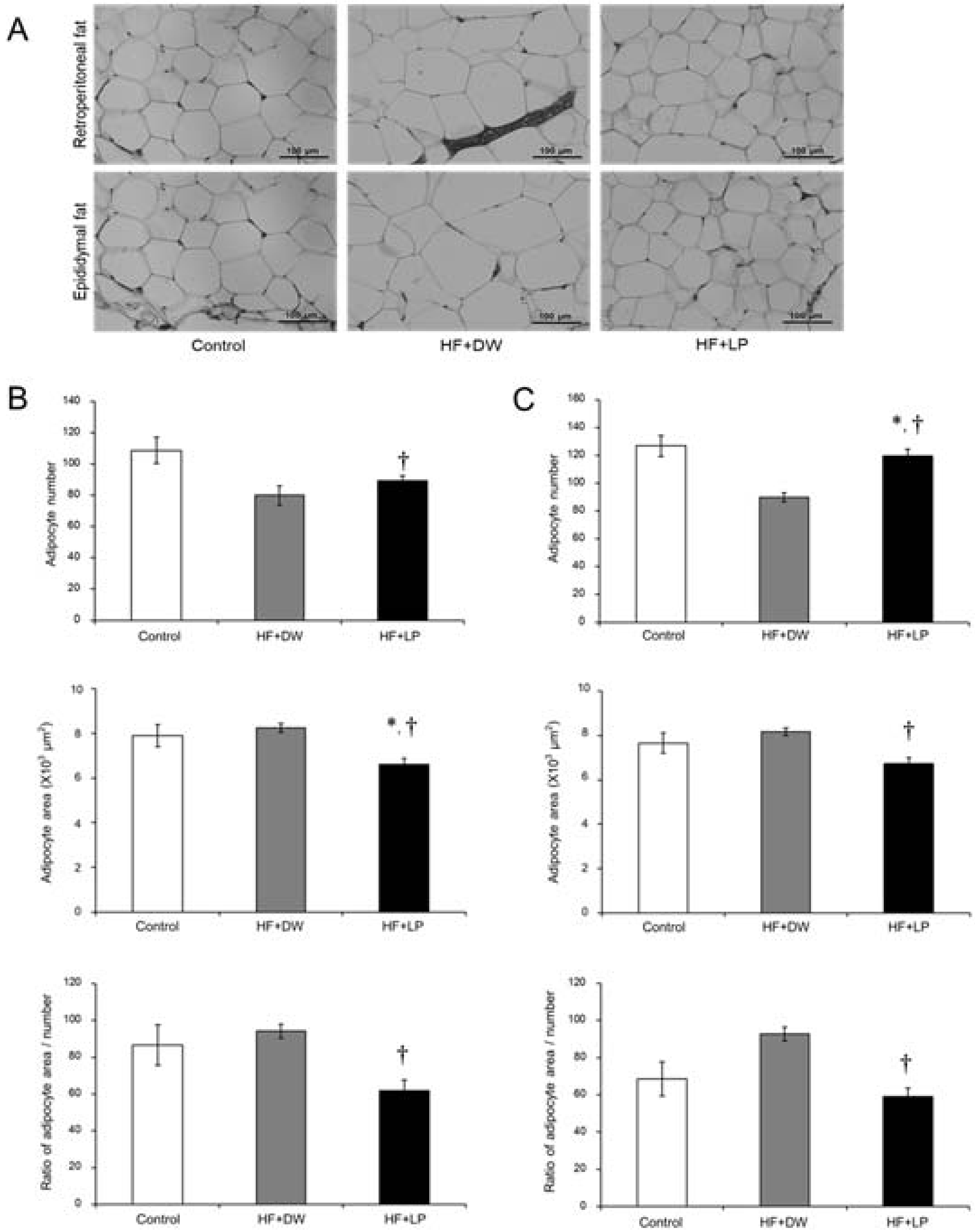

Figure 2. (A) The representative figure of retroperitoneal and epididymal adipocyte. The mean of adipocyte number, area and ratio of adipocyte area/number of retroperitoneal fat (B) and epididymal fat (C) compared among different three groups: control, HF + DW: a high-fat diet with distilled water, and HF + LP: high-fat diet with Luem Pua. Values are presented as mean ± S.E.M., *,□ Significant at $P < 0.05$ level compared with control group and HF ± DW.

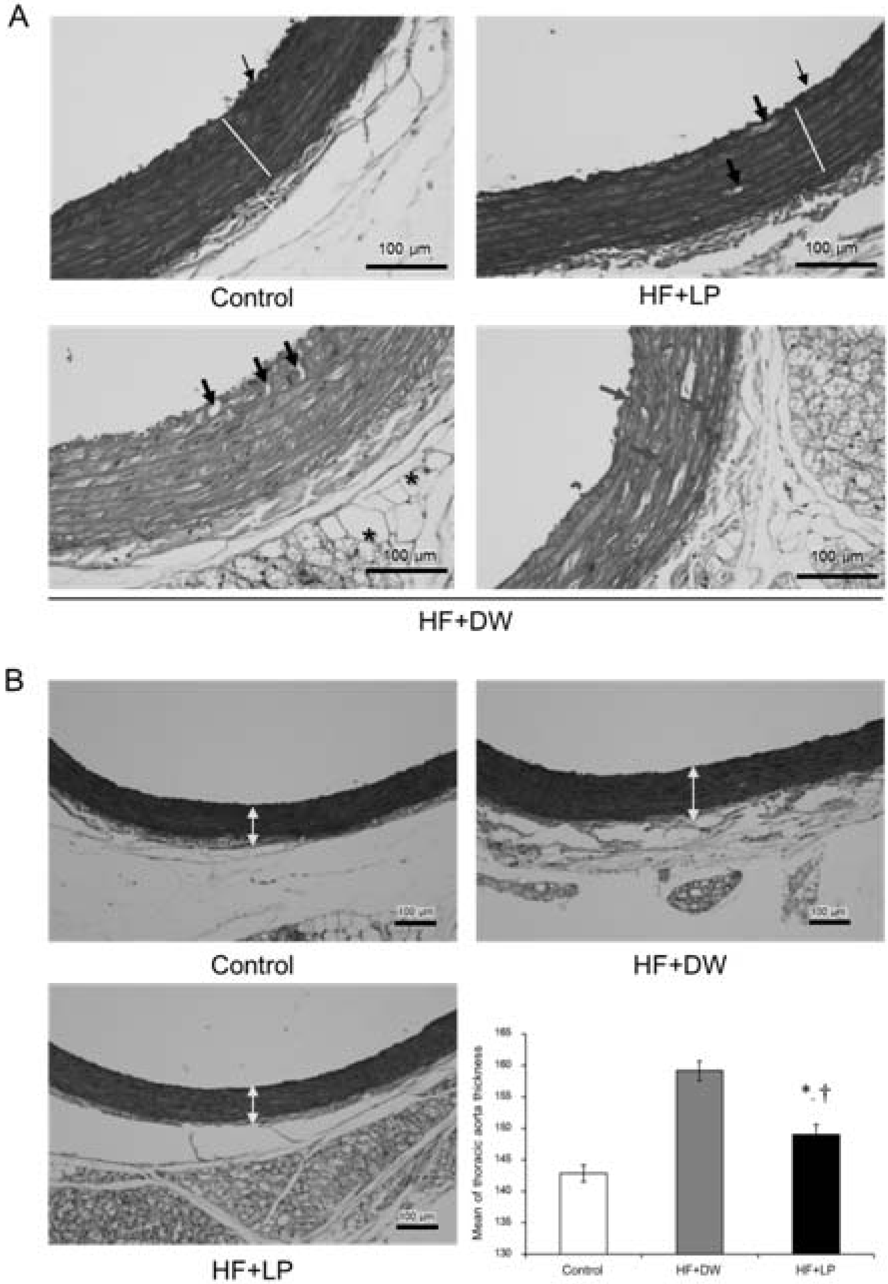

Figure 3. (A) Representative images of histomorphology of thoracic aorta in control, HF + DW: a high-fat diet with distilled water, and HF + LP: high-fat diet with Luem Pua. Normal aortic wall consists of *tunica intima* (thin arrow), *tunica media* (white line), and *tunica adventitia* (yellow line). Huge cytoplasmic vacuoles (thick arrow) represent in *tunica media* of HF group, which abundant in HF + DW. Irregular arrangement of smooth muscle cells (red arrow) in *tunica media*, and closely packed of adipose tissue (asterisk) to tunica adventitia are found in HF + DW. (B) Representative images of thoracic wall (yellow arrow) and mean of thoracic aorta thickness are compared among the three groups: control, HF + DW: a high-fat diet with distilled water, and HF + LP: high-fat diet with Luem Pua. Values are presented as mean ± S.E.M., *,□Significant at $P < 0.05$ level compared with control group and HF ± DW.

Discussion

In this study, high fat diet groups showed a significant increase of body weight than those in control group. We confirmed that 50% of high-fat diet in our experiment showed a tendency to increase body weight in the high-fat fed rat. Dosages of high fat diet have been reported in previous studies that it ranged from 10 kcal% to 60 kcal% fat to gain weight in animal model, and 60 kcal% fats was generally used to induce obesity in rodents as opposed to others.[15, 16] Decreasing of food intake and increasing of body weight in the high-fat fed rats resulted in the reverse correlation of these parameters, especially in those of the HF + LP fed rats. Decreasing of food intake in high-fat fed rats may due to increasing levels of the hormone leptin in those rats. Leptin is a major hormonal regulator of the energy requirement associated with eating behavior. Regarding leptin hormone and its mechanisms, it is secreted by accumulation of adipocytes in obese condition. There is the metabolic signaling to secrete anorexigenic peptides from hypothalamus. The function of these anorexigenic peptides such as proopiomelanocortin and corticotrophin-releasing hormone cause a reduction of food intake. [17-19]

Moreover, the high-fat diet groups showed increased abdominal organ weight, including the small intestine with mesentery, retroperitoneal and epididymal fat, which are the particular organs of fat accumulation in obese. [20, 21] Indeed, feeding of RS could reduce body weight and visceral organs weight. However, our results did not show the reduction of final body weight, visceral organs weight, serum glucose, total cholesterol, and HDL-cholesterol in rats fed with RS isolated from Luem-Pua glutinous rice. On the other hand, serum triglycerides, total cholesterol/HDL and triglycerides/HDL ratios were increased in the rats. This phenomenon in rats might be due to the lower level of RS was low and high carbohydrate composition in the samples because of limitations in the RS preparation. Previous studies have demonstrated that oral gavage containing with 6%, 13%, and 20% of RS for 5 weeks reduced weight gain and helped in loss of adipose tissue weight in rats. [11, 22, 23] In recent studies, addition of 10% RS in an oil- or lard-containing diet reduced serum cholesterol, triglycerides and glucose in rats. [14] Another study showed that 35.5% of RS feeding in high-fat fed rats reduced serum triglycerides level. [12] However, increasing serum triglycerides in high-fat diet feeding with RS might be caused by low ratio of triglycerides/HDL and decreasing of liver fat accumulation. Further research is needed to examine whether or not feeding rats with RS, using a high purified dose can reduce serum glucose, triglycerides, and lipid profiles. Alternatively, further research should investigate whether the effect of RS is on the lipid accumulation is in the liver or around the kidney.

In this study, the histology of adipocytes did not change in the high-fat diet groups compared with the control group. Particularly, the adipocyte area and ratio of adipocyte area/number of HF + LP were significantly higher than that in control and HF + DW. We suggest that RS may involve in the decrease of visceral adipocyte area via stimulation of the lipid metabolism pathway. The pathological condition of obesity is an excess of body fat, either by an increased number of adipocytes (hyperplasia) or by an increase of the size of adipocytes (hypertrophy). The adipocytes develop hyperplasia and hypertrophy due to chronic energy excess and cannot control proper energy storage. [20, 21, 24] RS feeding might stimulate lipid metabolism by inhibition of fatty acid synthase (FAS) activity and GLUT4 expression.[25] This control mechanism would decrease glucose uptake and total lipogenesis in adipocytes. [26-28] Furthermore, we suggest that RS is likely to prevent pathological changes and thickness of the thoracic aorta. A number of previous studies have reported that the thickening of *tunica intima* and *tunica media* is a characteristic of aging aorta and arteriosclerotic lesion. [29, 30] Either aberrant characteristic of pale cytoplasm and huge cytoplasmic vacuoles or thickening in the *tunica media* were found in the inflammatory response of vascular walls, hypertension, and cardiomyopathy. [31-34] This phenomenon may be explained that RS isolated from Luem-Pua glutinous rice may directly affect the accumulation of fat in the adipose tissue of the skin more than that of the internal organs such as the visceral fat or thoracic aorta. However, this study does not provide information how the thickening of the aorta can be reduced from high-fat diet plus RS. Future study should investigate the effect of RS on the expression of inflammatory cytokines or glycogen protein deposits in the aorta.

Conclusion

This study addressed specific protective effects of RS isolated from Luem-Pua glutinous rice on obesity as follows: 1) reduction of adipocytes size; 2) prevention of pathological changes; and 3) decreasing of thoracic aorta thickening in obese rats. According to these effects, we suggest that RS isolated from Leum-Pua glutinous rice might be further investigated to expand the supporting information for potential use as dietary supplements and development of therapeutic drug for obesity.

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