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Anti-Obesity Effect of Red Radish Coral Sprout Extract by Inhibited Triglyceride Accumulation in a Microbial Evaluation System and in High-Fat Diet-Induced Obese Mice

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Rhodosporidium toruloides, an oleaginous yeast, can be used as a fast and reliable evaluation tool to screen new natural lipid-lowering agents. Herein, we showed that triglyceride (TG) accumulation was inhibited by 42.6% in 0.1% red radish coral sprout extract (RRSE)-treated *R. toruloides*. We also evaluated the anti-obesity effect of the RRSE in a mouse model. The body weight gain of mice fed a high-fat diet (HFD) with 0.1% RRSE (HFD-RRSE) was significantly decreased by 60% compared with that mice fed the HFD alone after the 8-week experimental period. Body fat of the HFD-RRSE-fed group was dramatically reduced by 38.3% compared with that of the HFD-fed group.

Keywords: *Rhodosporidium toruloides*, red radish coral sprout, triglyceride, anti-obesity

In eukaryotes and several types of bacteria, neutral lipids, including triglycerides (TGs), are stored in lipid droplets (LDs) that serve as the energy reservoir of the cell [1, 2]. LDs are associated with oil production in microorganisms and plants [1]. However, in humans, they are often associated with metabolic diseases such as obesity, diabetes, atherosclerosis, and cancers [1, 2].

Preadipocytes such as 3T3-L1 have been traditionally used as in vitro cell models before in vivo testing for obesity research. Although the in vitro evaluation using cultivated cells is economical and convenient, this method can be disadvantageous owing to problems such as cell damage occurring during cell maintenance and storage [3, 4].

Oleaginous yeasts highly producing lipids are getting recognition in the field of microbial biodiesel [5] and also proposed as an in vitro model for obesity [6]. Among them, the red yeast *Rhodosporidium toruloides* is capable of accumulating lipids, such as TGs, at more than 70% of its dry cell biomass [7]. Previously, we reported *R. toruloides* as a novel in vitro tool to evaluate the TG reduction effects of potential anti-obesity food materials [4].

Radish (*Raphanus sativus*) possesses various biological properties, such as antimicrobial, antiviral, antioxidant, antitumor, antiplatelet, and immunological activities, intestine motility stimulation, and cardiovascular disease prevention [8]. In particular, red radish is used as a functional natural colorant because it is rich in anthocyanin pigments, which are known natural antioxidants [8−10].

In this study, we examined the inhibitory effect of the red radish coral sprout extract (RRSE) among anti-obesity candidates in TG accumulation, using the in vitro microbial evaluation system *R. toruloides,* and confirmed its anti-obesity activity in a diet-induced obesity mouse model.

The RRSE powder was kindly provided by Aenong Association (Korea). The extract powder was added to a yeast extract peptone dextrose (YPD) broth (Becton, Dickinson and Company, Sparks, USA) at a final concentration of 0.1%, 0.5%, and 1.0% (w/v). After culturing *R. toruloides*, TG was extracted from the cultures according to the method described by Lee [4]. The analysis of TG was determined using a TG assay kit (Asanpharm, Korea) according to the manufacturer's instructions.

Five-week-old C57BL/6J mice (19−20 g, male) were purchased from OrientBio (Korea). After 1-week adaptation

Table 1. Nutritional compositions of the diets.

	ND[a]		HFD[b]	
Product	gm%	kcal%	gm%	kcal%
Protein	20.0	20.3	26.2	20.0
Carbohydrate	64.0	63.9	26.3	20.0
Fat	7.0	15.8	34.9	60.0
Total	91.0	100.0	87.4	100
kcal/gm	3.9		2.24	
Ingredient	gm%	kcal%	gm%	kcal%
Casein, 80 Mesh	200.0	800.0	200.0	800.0
L-Cysteine	3.0	12.0	3.0	12.0
Corn starch	397.0	1590.0	0.0	0.0
Maltodextrin, 10	132.0	528.0	125.0	500.0
Sucrose	100.0	400.0	68.8	275.2
Cellulose, BW200	50.0	0.0	50.0	0.0
Soybean oil	70.0	630.0	25.0	225.0
Lard	0.0	0.0	245.0	2205.0
Mineral mix S10026	35.0	0.0	10.0	0.0
DiCalcium phosphate	0.0	0.0	13.0	0.0
Calcium carbonate	0.0	0.0	5.5	0.0
Potassium citrate, 1 H_2O	0.0	0.0	16.5	0.0
Vitamin mix V10001	10.0	40.0	10.0	40.0
Choline bitartrate	2.5	0.0	2.0	0.0
FD&C Blue	0.0	0.0	0.05	0.0
Total	1,000.0	4,000.0	773.85	4,057.0

[a]ND; Normal diet (AIN-95G). [b]HFD; High fat diet.

under a 12-h light/12-h dark cycle (temperature 22 ± 2°C, humidity 50 ± 5%), the mice were divided into three groups (10 mice per group). For 8 weeks, each group was fed different diets: (i) normal diet (ND), (ii) high-fat diet (HFD), and (iii) HFD with RRSE. RRSE was dissolved in sterilized drinking water at 1,000 mg/kg body weight and orally administered on a daily basis. The same volume of drinking water was orally administered to the mice in the ND and HFD groups. The mice were allowed free access to food (Table 1) and water during the 8-week experimental period. Food consumption and weight gain were measured daily and weekly, respectively. The body fat weight of each experimental group was measured photographically using dual energy X-ray absorptiometry (DEXA) (total-body scanner; InAlyzer Dual X-ray Absorptiometry; Medikors, Korea). All procedures were approved by the animal ethics committee of Wonkang University (Approval No. WKU15-117).

All results were expressed as the average and standard deviation using SPSS ver. 12 for Windows (SPSS Inc., USA).

Table 2. Effect of red radish sprout extract on inhibition of triglyceride (TG) accumulation in *Rhodosporidium toruloides*.

Sample	Wet cell mass (g)	Total lipid content (mg/g wet cell mass)	TG content (mg/dl)
Control	0.49 ± 0.03[b]	19.76 ± 3.53[a]	894.23 ± 85.71[a]
0.1% RRSE	0.62 ± 0.02[a]	18.10 ± 2.09[a]	513.30 ± 44.29[b]
0.5% RRSE	0.59 ± 0.08[a]	14.83 ± 1.37[a]	384.48 ± 34.06[c]
1.0% RRSE	0.60 ± 0.03[a]	16.12 ± 3.34[a]	346.06 ± 62.85[c]

Control, *R. toruloides* incubated in YPD medium without red radish sprout extract; 0.1%, 0.5%, and 1.0% RRSE, *R. toruloides* incubated in YPD medium with 0.1%, 0.5%, and 1.0% red radish sprout extract, respectively. Data are means ± standard deviations from three independent experiments. [a–c]Means with the different letters within the same column are significantly different at $p < 0.05$ by Duncan's multiple range test.

Data were analyzed by one-way ANOVA followed by Duncan's multiple range test for multiple comparisons. A difference of $p < 0.05$ was regarded as being statistically significant.

In the test for inhibitory effect of RRSE on TG accumulation in *R. toruloides*, the red yeast growth was not inhibited by treatment with RRSE (data not shown), and the wet cell mass was rather significantly increased compared with the control (Table 2). The TG content in the red yeast incubated in RRSE-including YPD medium was significantly decreased compared with the control, although the total lipid content was not different, demonstrating that RRSE can effectively lower TG accumulation in the red yeast in a concentration-dependent manner. The TG content in the yeast was significantly reduced to 0.1% RRSE concentration compared with the control group. Although 0.5% RRSE lowers more TG content than 0.1% RRSE does, 0.5% RRSE showed a slight decrease in the TG content by 14.4% compared with 0.1% RRSE.

On the basis of these in vitro results using the red yeast, the biological effect of RRSE was further evaluated using an in vivo mouse model. The HFD-induced obese mice were supplemented with 0.1% RRSE and their body weight, body fat weight, and serum TG concentration were measured.

Daily feed intake was significantly reduced in the HFD and HFD-RRSE groups compared with that in the ND group (Fig. 1A). Moreover, the feed efficiency of HFD group was significantly higher than that of the ND group (Fig. 1B); however, the efficiency of the HFD-RRSE group was not significantly different compared with that of the ND or HFD group (Fig. 1B). The weight of the HFD group after the 8-week experimental period was significantly increased by 26.6% compared with the ND group (27.8 ± 0.37 g), whereas that of the HFD-RRSE group was increased by

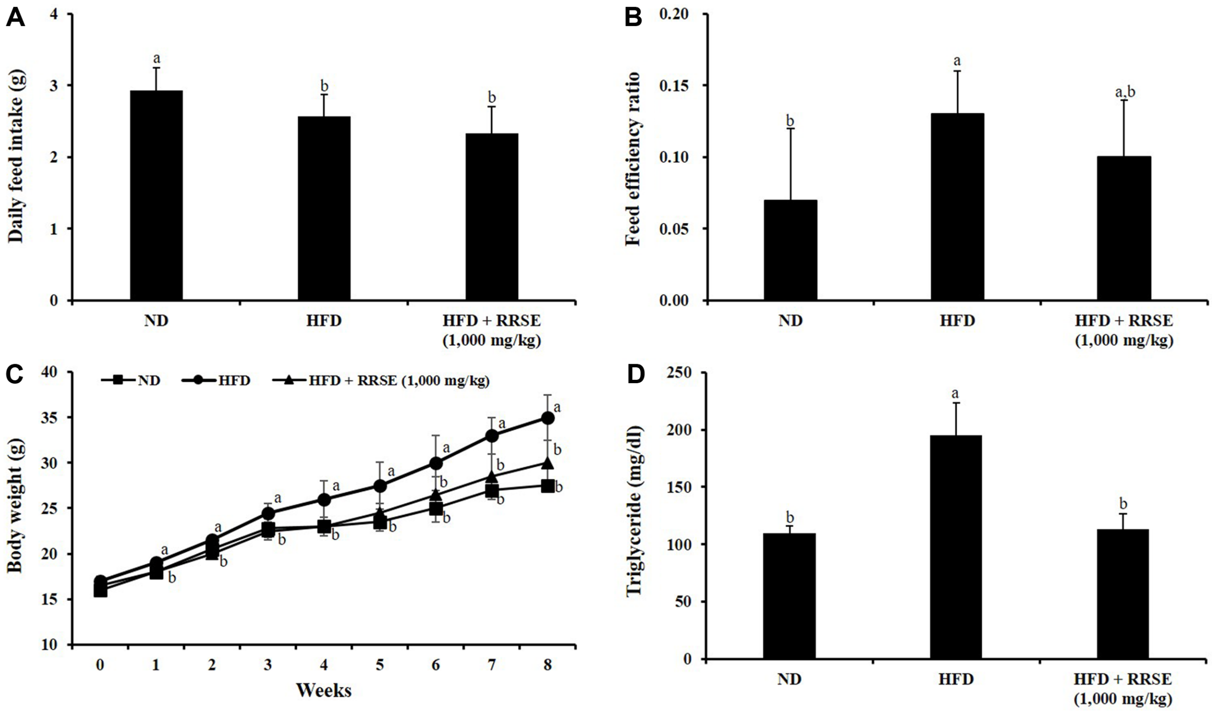

Fig. 1. Effect of red radish sprout extract supplementation on body weight and serum triglyceride (TG) level in HFD-fed C57BL/6J mice over a span of 8 weeks.

(**A**) Daily food intake; (**B**) Food efficiency ratio; (**C**) Weekly body weight measurements; (**D**) Serum TG level. Values with different letters are significantly different ($p < 0.05$) as determined by Duncan's multiple range test. ND, normal diet; HFD, high-fat diet; HFD + RRSE, HFD with red radish sprout extract.

10.5% compared with the ND group (Fig. 1C). However, there was no significant difference in the weights between the HFD-RRSE group and the ND group. In addition, the serum TG level of the HFD group was significantly increased compared with the ND group; however, the level of the HFD-RRSE group was markedly decreased to the level of the control group (Fig. 1D). Body fat weights in the HFD group and HFD-RRSE group were measured by DEXA scan (Fig. 2). The distribution of body fat was graphically visualized as high body fat, with dense fat significantly concentrated in the abdomen in the HFD group (Fig. 2A). The weight in the HFD group was increased by 25.6% compared with the ND group (Fig. 2B). In the HFD-RRSE group, the weight was increased by 15.8% compared with the ND group; it decreased by 38.3% compared with the HFD group (Figs. 2A and 2B).

In the previous study, we demonstrated that RRSE treatment of over 200 μg/ml could suppress the expression of adipogenic transcription factors (C/EBPα, PPARγ, and SREBP-1) and proteins (adiponectine, FAS, perilipin, and FABP4) in 3T3-L1 preadipocytes [11]. Vivarelli *et al*. [12] recently demonstrated that red radish Sango sprout, containing high levels of anthocyanins and isothiocynates that promote an anti-obesity effect, was effective in reducing the body weight of HFD-fed obese rats. Therefore, the anti-obesity effect of RRSE in HFD-fed obese mice may be attributed to the presence of compounds such as anthocyanins and isothiocynates.

In this study, we demonstrated the anti-obesity effect of RRSE first in the *R. toruloides* microbial evaluation system where TG accumulation was inhibited, and double-checked the effect in a HFD-fed obese mouse model, showing body weight and fat and serum TG content decreases. *R. toruloides* is a potential biotechnological microorganism that can be used as a molecular and genetic tools [13]. Specifically, this yeast can produce high amounts of lipids by using various carbon sources such as sugars (glucose, sucrose, starch, cellulose, *etc*.), glycerol, fatty acids (stearic acid, *etc*.), and raw materials (molasses, sugarcane, *etc*.). Moreover, the expression levels of TG-related genes, such as glycerol 3-phosphate dehydrogenase, fatty acid synthase, and diacylglycerol acyltransferase, associated with human adipocytes can be ascertained. Therefore, the yeast can be used as a fast and reliable screening or evaluation tool in novel approaches to

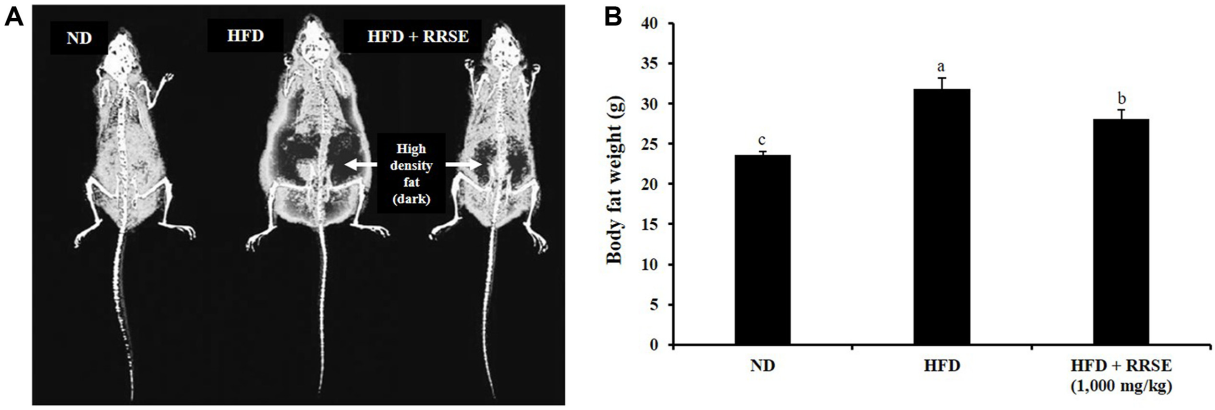

Fig. 2. Effect of red radish sprout extract supplementation on body fat weight in HFD-fed C57BL/6J mice.
(**A**) Body fat images of representative mice obtained by dual energy X-ray absorptiometry. (**B**) Body fat weight measurements during 8 weeks. Values with different letters are significantly different ($p < 0.05$) as determined by Duncan's multiple range test. ND, normal diet; HFD, high-fat diet; HFD + RRSE, HFD with red radish sprout extract.

select new favorable natural lipid-lowering agents that are directly applicable to in vivo experiments.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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