

## ORIGINAL ARTICLE

# Suppression of Feed Intake in Response to Rice Bran Oil Supplementation in Normal Rat

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## ABSTRACT

**Introduction:** Rice bran oil (RBO) is used in Asian countries as a daily dietary supplement. RBO is known in particular for its hypolipidemic effect. There has been increasing interest recently in the use of RBO as a means to maintain body weight and prevent obesity, though the mechanism of how this happens is still not well understood. We have investigated the effect of RBO on expression of genes that might influence energy homeostasis and feed intake. **Methods:** This study assessed Sprague-Dawley male rats at 12-weeks that were split into three groups over a 28-day period. A control group was fed a diet of standard rat chow, a standard group was fed standard rat chow with Orlistat (10.8 mg/kg bw/day), and a treatment group was fed standard rat chow with RBO (57.6 mg oryzanol/day). All supplementation was given by oral gavage. Possible adiposity was investigated through a histological analysis of adipocytes size measurement of intra-abdominal white adipose tissue in the rats. Changes in gene expression in the liver were determined by microarray. **Results:** The data suggest that RBO supplementation of a regular diet did not result in excess body weight and adiposity. A microarray analysis of the rats' livers found that RBO altered the expression of genes related to energy homeostasis and feeding behavior, by upregulating genes such as *Olr522*, *RGD1561231* and *Rgs16*. **Conclusion:** It is suggested that RBO supplementation can be used to maintain body weight by lowering appetite.

**Keywords:** Energy homeostasis, Liver, Microarray, Orlistat, Oryzanol

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## INTRODUCTION

Rice bran oil (RBO) is extracted from the chaff of rice (*Oryza sativa*) and is often used as cooking oil and in salads in some Asian countries. RBO contains a variety of compounds that have high antioxidant activity, including tocopherol, tocotrienol and oryzanols. Lately, researchers have been looking at ways to produce good-quality RBO that does not lose much of its free fatty acid content while being refined. Equipment is needed to retain the bioactive compounds in refined RBO, such as gamma-oryzanol, an unsaponifiable matter fraction of crude RBO that separates during RBO refining process (1, 2).

RBO is reported to have a role in health at a time when cardiovascular disease (CVD) is believed to be the most common cause of death (3,4). High blood cholesterol and diabetes are among the main risk factors for CVD, while RBO has been seen to improve lipid profile, blood glucose level, cancer, and muscular strength. Its popularity is based on nutritional benefits, primarily

through its ability to reduce cholesterol in persons with a high blood lipid profile (5). For someone who is normolipidemic, RBO can help maintain blood cholesterol at ideal levels (6). RBO's ability to lower blood cholesterol is comes from the unsaponifiable matter it contains, such as gamma oryzanol, and not its fatty acid composition (7). Research on rice bran as an anti-obesity treatment was carried out Recently in a study that found that when rats are fed a high-energy-density diet with rice bran, their body weight and adipocyte size reduce (8).

Many researchers have reported the various ways in which RBO's molecular mechanism can improve lipid profiles. Adding RBO into one's diet has the desirable effect of lowering blood lipid and also suppressing hyperinsulinemia in diabetic rats (9, 10). RBO's high gamma-oryzanol and gamma-tocotrienol content can increase cholesterol synthesis and catabolism, thus elevating the excretion of fecal neutral sterol and bile acid. This concomitantly leads to the hypocholesterolemia effect (9) or down-regulation of lipogenesis genes expression and differences in their metabolites (10). Unfortunately, little research has been done to reveal its anti-obesity properties at a molecular level.. Therefore, this study was conducted to investigate how energy homeostasis and feed intake were effected

by RBO supplementation, and find out which genes were involved.

## MATERIALS AND METHODS

### Animals and treatments

All animal experiment procedures were in accordance with the animal experiments guidelines, and were approved by the Animal Experimentation Ethics Committee, IPB University Veterinary Teaching Hospital. The rats were housed and handled under standard laboratory animal care procedures (temperature of  $22 \pm 2^\circ\text{C}$ , 12 hours dark/light cycle).

Male white Sprague Dawley rats (12 weeks old, body weight  $\pm 300$  g) purchased from Biopharmaca Research Centre (IPB University, Bogor, Indonesia) were housed at IPB University Veterinary Teaching Hospital. The rats were kept for seven days to adapt, and then divided into three groups (7 rats per group). The negative control group consisted of rats fed a standard rat chow diet (23% protein, 4% fat, energy 2,750 kcal/100g; Indonesia Formula Feed, Bogor, Indonesia). The positive control group comprised of rats that were fed a standard rat chow diet plus 10.8 mg/kg b.w. orlistat (Xenical, Roche, Switzerland). Each rat in the third group was fed a standard rat chow diet plus RBO (57.6 mg gamma-oryzanol/day; Oryza Grace, Thailand). The RBO dose was chosen based on Kustiyah et al. (11) and Damayanthi et al. (12). The RBO and orlistat were administered twice a day via oral gavage for 28 days, as the standard observation period for a clinical trial. The rats' body weight was measured once every week and its food intake was measured daily. After 28 days, all rats were made to fast overnight being sacrificed under anesthesia (mix of ketamine 75 mg/kg b.w. and xylazine 5 mg/kg b.w., intraperitoneal). Incision was made from rats' abdomen. The liver and white adipose tissue (WAT) were collected and weighed. The WAT was immediately treated for histological analysis, whereas the liver was stored in a freezer ( $-80^\circ\text{C}$ ) for further analysis.

### Histological analysis

Histological analysis was done on the epididymal WAT (13) which has been weighed and placed in 10% buffer neutral formalin fixative for 48 hours. The processed tissues were then embedded into paraffin blocks. Each section was cut three micrometers thick using rotary microtome (Reichert-Jung, Germany), then stained using haematoxylin and eosin (H&E). Mayer's haematoxylin was applied for eight minutes, lithium carbonate for 30 seconds, and Eosin for three minutes. Between each step, there was 30 seconds of washing with tap water. Each slide was examined under a light microscope (Olympus PM-10 AD, Japan) under the guidance of a pathologist, and pictures of adipose tissue were taken. The adipocytes size were measured using ImageJ (National Institutes of Health, Bethesda, Maryland, USA) on the basis of 200

adipocytes per animal, with clear-stained cells and no tissue contamination.

### Microarray analysis

The GeneChip® Rat Gene 2.0 ST Array (Affymetrix, United States) was used for microarray analysis at MacroGen Inc (South Korea). Three liver samples were examined from each group. After RNA isolation, only one sample from RBO group was suitable for further analysis. The microarray analysis processes were carried out according to previous work (14). Affymetrix® GeneChip Command Console® Software was used for exporting and analyzing array data. The robust multi-array average method was used to normalize the scanned reading. Changes in gene expression were calculated as log-ratio signal between intervention and control. Gene-enrichment and functional annotation analysis was carried out through Gene Ontology (geneontology.org), from which a list of significant probes was created. All data analysis and visualization of differentially expressed genes was conducted using R 3.3.2 (www.r-project.org). The top 10 upregulated and down-regulated genes were determined only for genes whose orthologs were already known.

### Statistical analysis

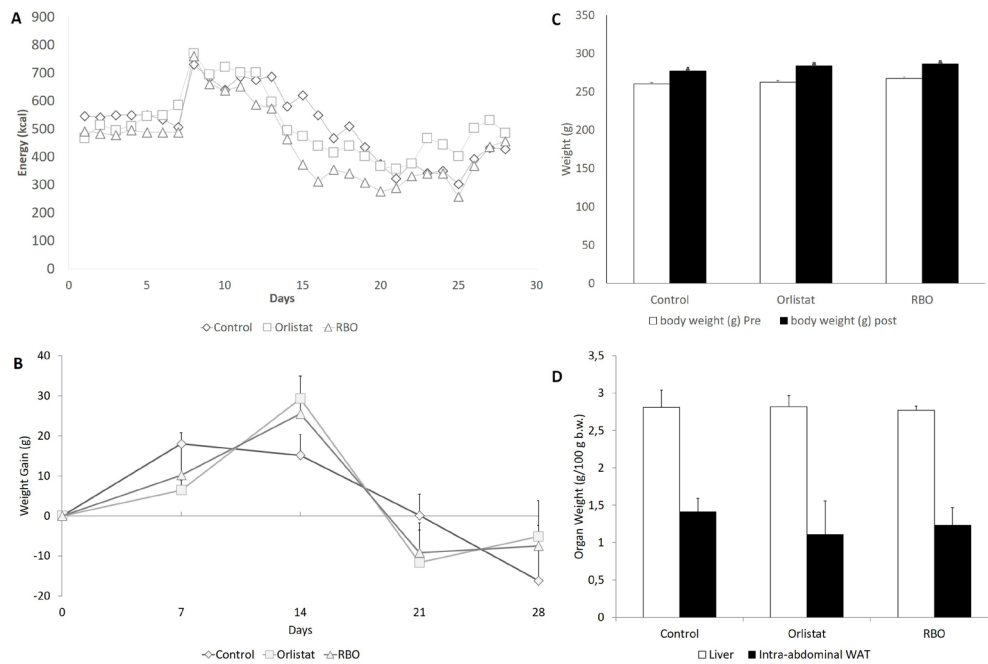
Statistical analysis was carried out by Student's t-test using GraphPad Prism. *P* value  $< 0.05$  was considered statistically significant. Data are represented as means  $\pm$  SEM.

## RESULTS

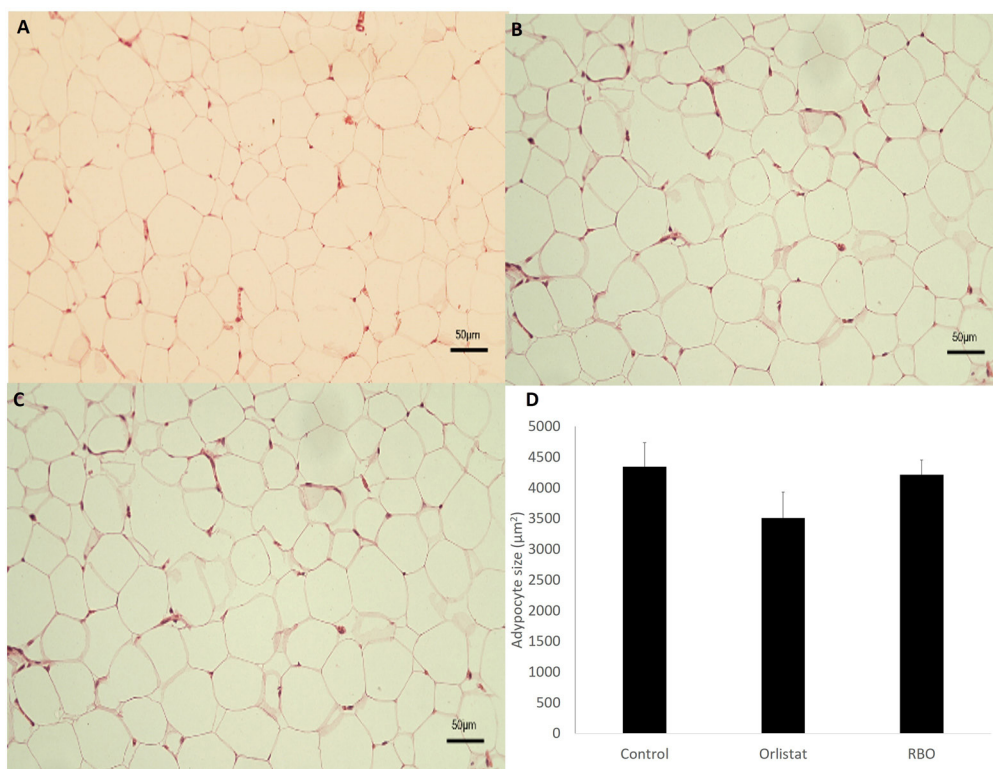
### Rat supplemented with Rice Bran Oil (RBO) did not gain excess weight and did not alternate adiposity

We observed that RBO group had a lower body weight than control (Figure 1A), though the difference was not significant. During the first two weeks, both RBO and orlistat groups showed remarkable body weight gain, which declined thereafter (Figure 1B). This decline after day 14 can be attributed to a decrease of food intake (Figure 1C). To further investigate whether RBO supplementation triggered adiposity in the liver and intra-abdominal WAT, we performed a histological analysis.

There was no difference between any of the group in terms of the main organs that store adipocyte (liver and intra-abdominal WAT) (Figure 1D). Furthermore, H&E staining of epididymal WAT showed no clinical abnormalities and sign of adiposity (Figure 2A-C). Adipocyte size quantification showed no significance difference between groups with  $4345.25 \pm 389.35 \mu\text{m}^2$ ,  $3507.16 \pm 423.70 \mu\text{m}^2$ , and  $4215.51 \pm 238.03 \mu\text{m}^2$  for control, orlistat, and RBO group, respectively (Figure 2D). RBO groups tended to have smaller adipocyte sizes than the control group. Altogether, the data suggests that RBO supplementation, in addition to a normal diet, did



**Figure 1: Growth performance of rats fed normal diet and supplemented either with water (control), Orlistat (standard), or RBO (test) for 28 days (n = 5-7 per group).** (A) Average daily calorie intake. (B) Bodyweight changes in rats. (C) Body weight before and after 28-days intervention. (D) Liver and Intra-abdominal WAT weight after 28 days. Error bars represent SEM. \* indicates significantly different according to Student t-test ( $p < 0.05$ ).



**Figure 2: Histological analysis.** H&E staining of epididymal fat of (A) control group, (B) Orlistat group, and (C) RBO groups after 28-days intervention.

not result in excess body weight gain.

### RBO supplementation affect genes related to sensory perception

To identify RBO's molecular mechanism that manages body weight, we performed a whole genome microarray analysis using Affymetrix GeneChip Rat Gene 2.0 ST

Array on the rats' livers. The mRNA expression of 29,489 probes was examined from one sample of liver from RBO group and three samples of liver from orlistat group. The expression of upregulated genes ( $\log_{2}FC > 2$ ) between the orlistat and RBO groups observed approximately 341 probes and 19 probes, respectively, while down-regulated genes ( $\log_{2}FC > 2$ ) had 43 and 124 probes,

respectively (Figure 3A). Based on the number of probes that are up- and down-regulated, it can be seen that orlistat and RBO works differently. Our analysis revealed that biological process with the highest significance counts were related to "neurological system process", "G-protein coupled receptor signaling pathway", and "sensory perception of chemical stimulus" (Figure 3C).

The most upregulated gene by RBO supplementation was Olf522, an olfactory receptor that relates to odorant molecules in the nose to activate the perception of scent (Table I). The olfactory receptor molecular function is among the top ten terms of GO functional analysis (Figure 3D). On the other hand, RT1-S2 is the most down-regulated genes (Table II). It is expected to be involved in antigen handling and the arrangement of endogenous peptide antigen.

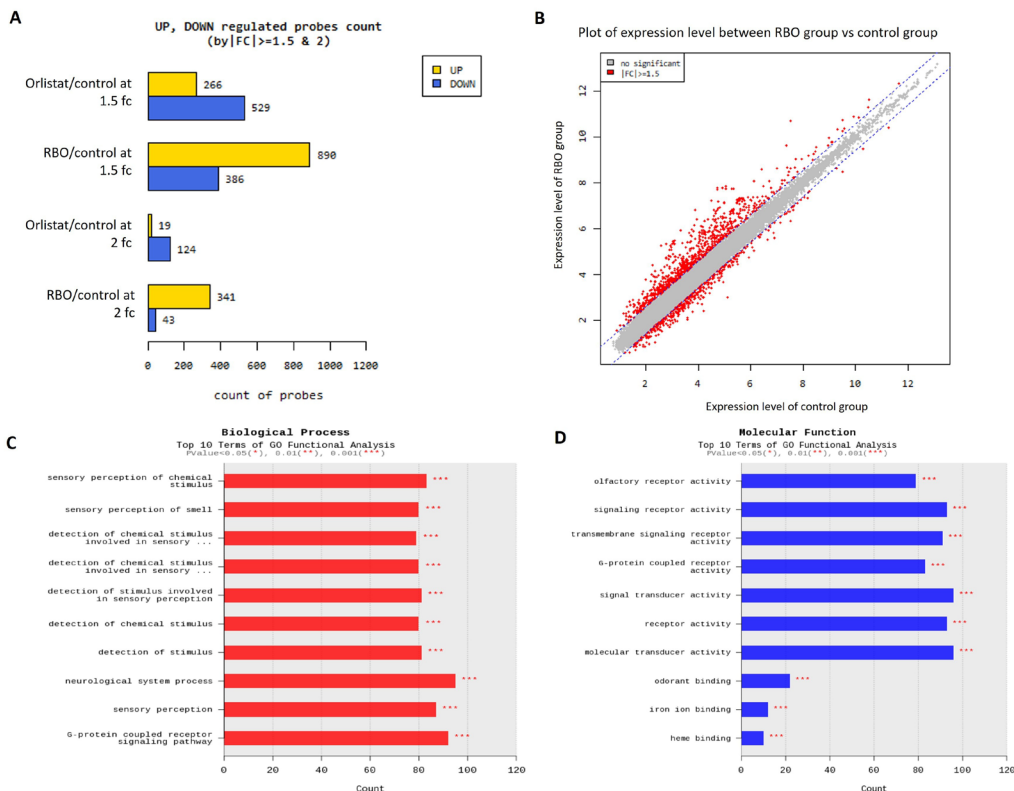
**DISCUSSION**

RBO is often used as dietary supplement and is seen as a safe alternative to prevent obesity, compared to drugs such as orlistat. Our data indicate that RBO prevented the possible weight gain, despite the extra energy in the oil itself. It is worth noting that the amount of calories ingested per day was lower, thus we cannot draw a conclusion over whether the effect is from bioactive component of RBO or limited calorie intake. However, RBO is rich in C18 fatty acids (15). It is known that long-chain fatty acid may reduce food intake. Previous

**Table I: Top 10 up-regulated genes**

Gene	Name	Predicted Function *)	Fold Change
Olf522	olfactory receptor 522	Involved in G protein-coupled receptor signaling pathway and sensory perception of smell.	9,30
RGD1561231	Similar to microtubule affinity-regulating kinase 4	Involved in intracellular signal transduction and microtubule cytoskeleton organization.	5,89
Rgs16	regulator of G-protein signaling 16	Involved in G protein-coupled receptor signaling pathway and positive regulation of GTPase activity.	4,49
MGC108823	similar to interferon-inducible GTPase	Involved in cellular response to interferon-beta and defense response.	4,35
RGD1561667	similar to putative protein kinase	Involved in intracellular signal transduction and protein phosphorylation.	3,96
Adh6	alcohol dehydrogenase 6 (class V)	Involved in ethanol oxidation; response to ethanol; and retinoid metabolic process.	3,87
RGD1564665	similar to RIKEN cDNA 4930555G01	Interact with indole-3-methanol	3,18
Scd1	stearoyl-CoA desaturase	Exhibits iron ion binding activity and stearoyl-CoA 9-desaturase activity. Involved in response to fatty acid and unsaturated fatty acid biosynthetic process	3,09
Smok2a	sperm motility kinase 2A	Involved in intracellular signal transduction and protein phosphorylation	3,06
Vnn3	vanin 3	Involved in pantothenate metabolic process	2,87

\*) based on NCBI gene data base



**Figure 3: Microarray analysis results.** (A) up-/down-regulated probes (fold change based). (B) Plotted a scatter plot of expression level between each comparison. Top 10 terms of Gene Ontology Enrichment Analysis result were described by Bar graph for (C) Biological Process and (D) Molecular Function.



**Table II: Top 10 down-regulated genes**

Gene	Name	Predicted Function *)	Fold Change
RT1-S2	RT1 class Ib, locus S2	Involved in several processes, including antigen processing and presentation of endogenous peptide antigen via MHC class I via ER pathway, TAP-independent; antigen processing and presentation of endogenous peptide antigen via MHC class Ib; and positive regulation of T cell mediated cytotoxicity.	-2,67
Olr832	olfactory receptor 832	Involved in G protein-coupled receptor signaling pathway and detection of chemical stimulus involved in sensory perception of smell.	-2,65
Prdm5	PR/SET domain 5	Involved in several processes, including cellular response to leukemia inhibitory factor; histone modification; and negative regulation of transcription by RNA polymerase II.	-2,47
Acmsd	amino-carboxy-muconate semialdehyde decarboxylase	Exhibits aminocarboxymuconate-semialdehyde decarboxylase activity. Involved in aging and tryptophan catabolic process	-2,47
RGD1563231	similar to immunoglobulin kappa-chain VK-1	Involved in immune response and immunoglobulin production.	-2,28
Acnat1	acyl-coenzyme A amino acid N-acyl-transferase 1	unknown	-2,23
Olr184	olfactory receptor 184	Involved in G protein-coupled receptor signaling pathway and detection of chemical stimulus involved in sensory perception of smell.	-2,12
Bst1	bone marrow stromal cell antigen 1	Involved in several processes, including positive regulation of B cell proliferation; regulation of neutrophil chemotaxis; and regulation of peptidyl-tyrosine phosphorylation.	-2,03
Mir328	microRNA 328	Involved in long-term synaptic potentiation.	-2,03
Abcg2	ATP binding cassette subfamily G member 2	Exhibits cytoskeletal protein binding activity and transmembrane transporter activity. Involved in several processes, including cellular response to lipid; embryonic process involved in female pregnancy; and urate salt excretion	-2,02

\*) based on NCBI gene data base

studies have shown that emulsion enriched with linoleic acid (C18:2) suppresses food consumption in humans, helping them concomitantly maintain their body weight (16; 17). Although these results do not exclude a role for gamma-oryzanol in reducing food intake, fatty acid composition of RBO seems a much more plausible mechanism for appetite suppression in rats.

The amount of fat ingested in the RBO group was the greatest due to the supplementation, though it seems that not all the fats has been absorbed. Histological analysis revealed that the RBO group tended to have smaller adipocyte sizes than the control group. This result is in line with a previous study that demonstrated how RBO supplementation can avert the growth of WAT while also boosting lipid metabolism in mice (18). Moreover, the gamma-oryzanol in RBO influences WAT size reduction and inhibits fat formation (19). Distribution of body fat is associated with liver fat content (20), and the

liver weights in the RBO group was similar to those of the control group, indicating that RBO supplementation does not induce adiposity, despite the additional fat intake.

We then took our investigation to a molecular level by using microarray to elucidate possible mechanisms for how RBO may act as anti-obesity treatment. Orlistat was used as standard because it is widely used to prevent lipid absorption, and consequently leads to weight reduction (21). However, it seems RBO and orlistat have different mechanisms, as seen in Figure 3A. The number of genes that were upregulated were different for each treatment (19 for orlistat and 341 for RBO). This is interesting because it has been suggested that gamma-oryzanol from RBO might prevent fatty acid absorption in the small intestine (22; 23). Future studies are needed to validate the current finding as the number of samples used for microarray analysis is limited.

Biological processes that were identified through a GO Functional Analysis in this study are dominated by sensory perception. Sensory perception of smell appeared predominantly after RBO supplementation. A previous study found that sense of smell has an effect on the metabolic pathway of hunger and satiety (24). Moreover, olfactory processing serves as a link between appetite, food reward, and metabolism (25). Based on an analysis of top 10 terms for molecular function, olfactory receptor activity occurs significantly.

According to a microarray analysis, Olr522 gene that encode for olfactory receptors is the most upregulated genes in rats' livers. While Olr832 and Olr184, which also have a sensory perception functions, are among the top 10 downregulated genes. Although the exact regulatory link among olfactory receptor genes is still unknown, several studies have attributed the role of olfactory receptors on appetite regulation (26-28). RGD1561231 and Rgs16 have been investigated for their roles on energy homeostasis related to feeding behavior (29; 30). RGD1561231, which is similar to microtubule affinity-regulating kinase 4 (MARK4), was shown to be a factor regulating AMP-activated protein kinase (AMPK) activity (29). While, Rgs16 is expressed in periportal hepatocytes to maintain body weight homeostasis through fatty acid oxidation and glucose production (30). Conceivably, suppressive effect of appetite from olfactory-receptors-related genes leads to low adiposity. Overall, our data indicate that RBO supplementation might influence rat appetite and energy homeostasis.

## CONCLUSION

We showed that supplementation of RBO in addition to normal diet in rats might have a beneficial effect on maintain body weight by reducing appetite. Reduced feed intake preventing excess fats stored in white adipose tissue. In this way, olfactory-receptors-related

genes might be a novel target gene to protect against obesity.

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