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A Rice Bran Oil Diet Improves Lipid Abnormalities and Suppress Hyperinsulinemic Responses in Rats with Streptozotocin/Nicotinamide-Induced Type 2 Diabetes

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Summary **The aim of this study was to determine the effects of rice bran oil (RBO) on lipid metabolism and insulin resistance in rats with streptozotocin/nicotinamide-induced type 2 diabetes mellitus (T2DM). Rats were divided into two groups: the control group (15% soybean oil, contains 0 g γ-oryzanol and 0 g γ-tocotrienol/150 g oil for 5 weeks) and the RBO group (15% RBO, contains 5.25 g γ-oryzanol and 0.9 g γ-tocotrienol/150 g oil for 5 weeks). Compared with the control group, the RBO group had a lower plasma nonesterified fatty acid concentration, ratio of total to high-density-lipoprotein cholesterol, hepatic cholesterol concentration, and area under the curve for insulin. The RBO group had a higher high-density-lipoprotein cholesterol concentration and greater excretion of fecal neutral sterols and bile acid than did the control group. RBO may improve lipid abnormalities, reduce the atherogenic index, and suppress the hyperinsulinemic response in rats with streptozotocin/nicotinamide-induced T2DM. In addition, RBO can lead to increased fecal neutral sterol and bile acid excretion.**

***Key Words*: rice bran oil**, **γ-oryzanol**, **γ-tocotrienol, type 2 diabetes, insulin resistance**

Introduction

The prevalence of type 2 diabetes mellitus (T2DM) has increased greatly in the past few years and a still greater increase is foreseen in the next few years [1]. For this reason, T2DM is considered to be one of the epidemics of the new century. Persons with T2DM have an increased risk of mortality, primarily because of cardiovascular disease (CVD) [2, 3]. An important risk factor for the development of CVD [4] is dyslipidemia, and randomized controlled studies have shown that a lipid-lowering treatment reduces the risk of CVD and death [5]. This combination of abnormalities—

elevated blood levels of triglycerides (TGs), low levels of high-density-lipoprotein cholesterol (HDL-C), and relatively normal levels of low-density-lipoprotein cholesterol (LDL-C) carried in small, dense, cholesterol-poor LDL particles—is known as "diabetic dyslipidemia." Significant evidence supports a key role for insulin resistance, which is a central pathophysiologic feature of T2DM in the development of diabetic dyslipidemia [6].

Many aspects of the diet (carbohydrate, fat, fiber, vitamins, and alcohol) are considered to be important in the modulation of insulin resistance; however, in the past few years, more attention has been given to the ability of the quality of dietary fat, independent of the total amount, to influence insulin sensitivity and to increase the risk of T2DM [7]. Rice bran oil (RBO) is recommended for the treatment of hyperlipoproteinemia in humans because plasma total cholesterol (TC), LDL-C, and TG concentra-

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tions decrease when this oil is added to the diet [*8–10*]. A hypolipidemic response characterized by decreases in serum concentrations of TG, TC, very-low-density lipoprotein cholesterol (VLDL-C), and LDL-C has been associated with feeding RBO to rats [*11*, *12*]. Rice bran also attenuates hyperglycemia in humans with diabetes mellitus [*13*]. Soluble components of rice bran lowered fasting glucose concentrations by 29% and 33%, respectively, in subjects with type 1 diabetes mellitus and T2DM [*13*].

In Taiwan, there has been a 5-fold increase in mortality from DM-related complications in the past 20 years. T2DM is associated with a high incidence of complications, such as retinopathies, glomerulopathies, and vascular complications. However, few studies have investigated the effect of RBO consumption in the T2DM model. Therefore, the present study was conducted to determine the effects of RBO consumption on lipid metabolism and insulin resistance in rats with streptozotocin (STZ)/nicotinamide-induced T2DM.

Materials and Methods

Animals and diets

Sixteen male Wistar rats aged 6 weeks with a body weight (BW) of 200 ± 10 g were obtained from the Animal Center of Taiwan University Medical College, Taipei, Taiwan. Rats were housed individually in wire-bottomed stainless steel cages in an air-conditioned room ($21 \pm 2°C$, 50–70% relative humidity) with a 12-h light-dark cycle and free access to the basal diet and water for 2 weeks before T2DM was induced. Diabetes was induced with an intraperitoneal injection of STZ (45 mg/kg BW), which was followed 15 min later by an injection of nicotinamide (200 mg/kg BW). After 2 days, this step was repeated using the modified Masiello method [*14*]. Nicotinamide and STZ were freshly prepared in a 0.9% (wt:v) sodium chloride solution. Rats were considered diabetic when their fasting plasma glucose concentration was greater than 10 mmol/l 14 days after the last induction date. At this time, baseline blood samples were collected from the tail vein of the rats after anesthetization with ether gas. Fatty acid composition, areas under the curve (AUCs) for glucose and insulin, and plasma glucose, insulin, TG, nonesterified fatty acid (NEFA), and cholesterol concentrations, were not significantly different from those of the diabetic rats at baseline. The rats were then fed the experimental diets.

Diabetic rats were divided into a control or RBO group ($n = 8$ per group) and were fed cholesterol-free diets for 5 weeks. The high-fat diets were a modified AIN-93M diet containing 150 g of fat, 590 g of cornstarch, 200 g of casein, and 10 g of α-cellulose as fiber/kg diet. Choline, cysteine, minerals, and vitamins were added as described in AIN-93M [*15*]. The experimental diet for the control group contained 15% soybean oil. The experimental diet for the RBO group

Table 1.   Composition of experimental diets[1,2]

	C	RBO
	\(g/kg diet)	
Cornstarch	449.5	449.5
Casein	200	200
Sucrose	100	100
Soybean oil	150	—
Rice bran oil	—	150
Fiber	50	50
Mineral mix	35	35
Vitamin mix	10	10
L-cystine	3	3
Choline bitartrate	2.5	2.5
γ-oryzanol (g/150 g oil)	—	5.25[3]
γ-tocotrienol (g/150 g oil)	—	0.9

[1] C: control group; RBO: rice bran oil group.
[2] Modified AIN-93M (American Institute of Nutrition, 1993).
[3] Rice bran oil content 0.035 g per gram.

contained 15% RBO in place of soybean oil. RBO was extracted by using the supercritical CO_2 fluid extraction method [*16*]. Each gram of RBO contained 0.035 g of γ-oryzanol and 0.006 g of γ-tocotrienol. The 15% RBO diet contained 5.25 g of γ-oryzanol and 0.9 g of γ-tocotrienol in 150 g RBO/kg diet (Table 1). All animal experimental procedures followed published guidelines [*17*] and were approved by the Institutional Animal Care and Use Committee of Taipei Medical University, Taipei, Taiwan.

After consuming the diets for 5 weeks, the diabetic rats were deprived of food overnight (12 h), after which they were anesthetized with ether and killed by exsanguination from the abdominal aorta. Blood was centrifuged at $1200 \times g$ at 4°C for 10 min, and the plasma was collected. The livers of all rats in both groups were removed. Fecal samples of the rats in each group were obtained at the end of the experimental period. All samples were frozen at 70°C until analysis.

Analysis

RBO and soybean oil were analyzed for γ-tocotrienol and γ-oryzanol by high-performance liquid chromatography (HPLC) using a method described previously [*18*]. The HPLC system consisted of a Hitachi L-2000 pump equipped with a Hitachi AS-2000 autosampler and a Hitachi L-7455 diode array detector (Hitachi, Ltd., Tokyo, Japan). A C_{18} normal-phase chromatography column (5 SL-II, 4.6×250 mm, 5 mm; Cosmoscil, Nacalai Tesque) was used. The flow rate was set at 1.0 mL/min, and the wavelength of the detector was set at 295 nm for the detection of γ-tocotrienol and γ-oryzanol. The mobile phase was hexane:isopropanol (99:1, v:v). The percentages of fatty acids in the RBO and

soybean oil were analyzed by gas chromatography [19]. The percentages of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) in the soybean oil were 16%, 25%, and 59%, respectively. The percentages of SFA, MUFA, and PUFA in RBO were 18%, 42%, and 40%, respectively (Table 2). The plasma glucose, TG, TC, HDL-C, LDL-C, and NEFA concentrations were determined spectrophotometrically using a glucose kit, TG kit, cholesterol kit, LDL-C kit, and NEFA kit, respectively (Randox, Antrim, United Kingdom). The plasma insulin concentration was measured by using a rat insulin enzyme-linked immunosorbent assay kit (Mercodia, Uppsala, Sweden). TGs and cholesterol from liver samples were extracted [20] and measured using a TG kit and a cholesterol kit (Randox, Antrim, United Kingdom). The total fatty acid composition of plasma and hepatic lipids was quantified using a one-step direct transesterification procedure carried out in a 4:1 methanol:hexane solution with acetyl chloride in all steps, as previously described [21]. The fatty acids were analyzed by gas chromatography with a G-3000 chromatograph (Hitachi Ltd, Tokyo, Japan) with flame ionization detection. Separations were performed on a Stabilwax-DA capillary column (30 m × 0.53 mm i.d.; film thickness, 0.5 μm; RESTEK). Individual fatty acids in the plasma and liver were calculated using 17:0 fatty acid as an internal standard. Identification was based on the retention time. Individual fatty acid levels were expressed as a percentage of total fatty acids. Because the amount of the fecal sample for individual rats in the same group was insufficient to analyze neutral sterols and bile acids, fecal

samples were pooled in the same group, divided into 5 equal portions, freeze-dried, and ground. Neutral sterols and bile acids were extracted and determined [21] using cholesterol and bile acids kits (Randox, Antrim, United Kingdom).

Intraperitoneal glucose tolerance test (IPGTT)

At week 0 and week 5, diabetic rats were deprived of food overnight for 12 h and injected intraperitoneally with glucose (0.5 mg/kg BW). Venous blood samples were then collected at 0, 15, 30, 60, 90, 120, and 180 min for the measurement of plasma glucose and insulin. Insulin resistance was estimated by the product of the AUCs for glucose and insulin as previously used by Cortez *et al.* [22].

Statistical analysis

Values were expressed as means ± standard deviations (SDs). Comparisons of data from two groups were conducted by student's *t* test by using SAS statistical software (version 8.2; SAS Institute Inc., Cary, NC). Differences were considered significant at *p*<0.05.

Results

Weight gain and food intake

The daily food intake during the experimental period did not differ between groups, and weight gain was also unaffected by the diet. No side effects, such as diarrhea or death, occurred in rats fed the experiment diet, and no rats died as a result of T2DM being induced by the STZ injections (Table 3).

Plasma glucose, insulin concentrations, and insulin sensitivity

After 5 weeks of treatment, the plasma glucose and insulin concentrations of rats did not differ between the groups (Table 4). AUCs for glucose and insulin are a measure of insulin sensitivity, and the AUC for glucose did not differ between groups. The AUC for insulin was signifi-

Table 2.   Fatty acid composition of experimental oils[1,2]

	Soybean oil	Rice bran oil
	(percent to total fatty acids)	
C14: 0	0.1 ± 0.0	0.4 ± 0.0*
C16: 0	11.4 ± 0.0	17.3 ± 0.5*
C18: 0	3.8 ± 0.2	—
C20: 0	0.3 ± 0.1	0.5 ± 0.1*
C22: 0	0.4 ± 0.0	0.2 ± 0.0*
ΣSFA[3]	16.0 ± 0.2	18.3 ± 0.3
C16: 1	—	0.1 ± 0.0
C18: 1	24.8 ± 0.5	42.0 ± 0.1*
ΣMUFA[3]	24.8 ± 0.5	42.1 ± 0.2*
C18: 2	53.0 ± 0.3	38.5 ± 0.2*
C18: 3	6.2 ± 0.2	1.2 ± 0.2*
ΣPUFA[3]	59.2 ± 0.3	40.0 ± 0.2*

[1] Values are means ± SD, *n* = 3.

[2] * means there is significant difference between soybean oil and rice bran oil (*p*<0.05).

[3] SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

Table 3.   Food intake, body weight, weight gain, liver weight, relative liver weight, epididymal fat weight, relative epididymal fat weight in diabetic rats after 5-week experimental diets[1,2]

	C	RBO
Food intake (g/day)	20.0 ± 1.9	19.3 ± 1.8
Weight (g)	471.3 ± 51.2	424.9 ± 32.1
Weight gain (g)	74.3 ± 20.0	63.2 ± 17.8
Liver weight (g)	11.9 ± 1.7	11.83 ± 1.2
Relative liver weight (%)	2.5 ± 0.2	2.7 ± 0.1
Epididymal fat weight (g)	13.1 ± 2.6	10.2 ± 2.0
Relative epididymal fat weight (%)	2.8 ± 0.4	2.4 ± 0.4

[1] C: control group; RBO: rice bran oil group.

[2] Values are means ± SD, *n* = 8.

Table 4. Plasma and hepatic biomarker in diabetic rats after 5-week experimental diets[1–3]

	C	RBO
Glucose (mg/dL)	192.1 ± 24.7	199.7 ± 18.2
Insulin (ug/L)	2.09 ± 0.73	2.29 ± 0.92
AUC$_{glucose}$[4] (mg × min/dL)	49734 ± 9480	49285 ± 6836
AUC$_{insulin}$[4] (μg × min/L)	290.4 ± 65.0	218.7 ± 65.9*
TC[5] (mg/dL)	77.5 ± 12.7	79.6 ± 5.0
LDL-C[5] (mg/dL)	18.7 ± 6.5	18.0 ± 5.9
HDL-C[5] (mg/dL)	53.7 ± 3.2	61.1 ± 2.9*
TC/HDL-C	1.5 ± 0.3	1.3 ± 0.0*
Triglyceride (mg/dL)	61.0 ± 18.7	59.3 ± 15.3
NEFA (mmol/L)	0.70 ± 0.07	0.58 ± 0.09*
Triglyceride (mg/g liver)	948.1 ± 67.0	929.7 ± 34.7
Cholesterol (mg/g liver)	159.7 ± 28.0	129.3 ± 17.5*
Neutral sterol (mg/g feces)	5.09 ± 0.15	15.03 ± 1.71*
Bile acid (μg/g feces)	0.80 ± 0.02	2.88 ± 0.17*

[1] C: control group; RBO: rice bran oil group.
[2] Values are means ± SD, $n = 8$.
[3] * means there is significant difference between control group and RBO group ($p<0.05$).
[4] AUC$_{glucose}$: area under the curve for glucose; AUC$_{insulin}$: area under the curve for insulin.
[5] TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; NEFA: non-esterified fatty acid.

Table 5. Plasma fatty acid compositions in diabetic rats after 5-week experimental diets[1–3]

	C	RBO
	(percent to total fatty acids)	
C14: 0	—	—
C16: 0	22.1 ± 0.6	25.0 ± 1.1*
C18: 0	16.7 ± 2.0	16.8 ± 1.8
ΣSFA[4]	36.9 ± 0.8	41.1 ± 2.5*
C16: 1	1.1 ± 0.2	1.1 ± 0.2
C18: 1	7.6 ± 1.7	13.4 ± 2.7*
ΣMUFA[4]	8.4 ± 1.7	14.8 ± 2.7*
C18: 2	20.9 ± 2.7	16.1 ± 2.4*
C18: 3	0.9 ± 0.2	—
C20: 4	28.2 ± 3.2	24.3 ± 7.9
C22: 6	3.2 ± 0.8	3.3 ± 0.3
ΣPUFA[4]	52.5 ± 2.0	43.7 ± 5.8*

[1] C: control group; RBO: rice bran oil group.
[2] Values are means ± SD, $n = 8$.
[3] * means there is significant difference between control group and RBO group ($p<0.05$).
[4] SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

cantly lower in the RBO group than in the control group ($p<0.05$) (Table 4).

Plasma cholesterol concentrations
After 5 weeks of treatment, plasma TC and LDL-C concentrations did not differ between the two groups. The HDL-C concentration was higher in the RBO group than in the control group. The TC/HDL-C ratio was lower in the RBO group than in the control group (Table 4).

Plasma TG and NEFA concentrations
Plasma TG concentrations did not differ between the groups. But the plasma NEFA concentration was significantly lower in the RBO group than in the control group after 5 weeks of treatment (Table 4).

Hepatic cholesterol and TG concentrations
The hepatic TG concentration did not differ between the two groups after 5 weeks of treatment. The hepatic cholesterol concentration was lower in the RBO group than in the control group (Table 4).

Fecal neutral sterol and bile acid contents
The fecal neutral sterol and bile acid contents were significantly higher in the RBO group than in the control group at the end of week 5 (Table 4).

Plasma and hepatic fatty acid composition
After 5 weeks of treatment, plasma and hepatic fatty acid compositions were consistent with the composition of the RBO. Total SFAs and MUFAs in plasma and liver were higher in the RBO group than in the control group. Total PUFAs in plasma and liver were lower in the RBO group than in the control (Tables 5 and 6).

Discussion

Individuals with T2DM have an increased risk of mortality, primarily because of CVD [*2*, *3*]. An important risk factor for the development of CVD [*4*] is dyslipidemia, and randomized controlled studies have shown that lipid-lowering treatments reduce the risk of CVD and death [*5*]. The present study showed that an RBO diet containing γ-oryzanol and γ-tocotrienol maintained plasma glucose concentrations; lowered the AUC for insulin, plasma NEFA concentrations, the TC/HDL-C ratio, and the hepatic cholesterol concentrations; increased the HDL-C concentration; and increased the fecal excretion of bile acids and neutral sterols in diabetic rats fed the RBO diet compared with rats fed a soybean oil diet that served as control and contained no γ-tocotrienol or γ-oryzanol. Thus, a diet containing RBO may improve lipid abnormalities and

Table 6.   Hepatic fatty acid compositions in diabetic rats after 5-week experimental diets[1–3]

	C	RBO
	(percent to total fatty acids)	
C14: 0	0.5 ± 0.0	0.5 ± 0.1
C16: 0	21.2 ± 0.7	24.7 ± 1.6*
C18: 0	14.8 ± 2.4	16.3 ± 2.8
ΣSFA[4]	36.4 ± 2.8	41.5 ± 3.2*
C16: 1	0.7 ± 0.2	0.8 ± 0.2
C18: 1	13.4 ± 2.4	21.2 ± 3.9*
ΣMUFA[4]	14.0 ± 3.3	22.0 ± 4.0*
C18: 2	28.4 ± 2.5	18.2 ± 3.0*
C18: 3	1.2 ± 0.2	0.3 ± 0.1*
C20: 4	15.6 ± 2.2	16.8 ± 2.5
C22: 6	4.6 ± 1.3	4.3 ± 1.5
ΣPUFA[4]	49.6 ± 1.6	36.5 ± 5.0*

[1] C: control group; RBO: rice bran oil group.

[2] Values are means ± SD, $n = 8$.

[3] * means there is significant difference between control group and RBO group ($p<0.05$).

[4] SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

reduce the increased atherogenic index in rats with STZ/nicotinamide-induced T2DM.

In various animal species, several syndromes resembling type 2 DM either occur spontaneously or can be induced experimentally by different procedures [23]. None of these syndromes is able to reproduce the complexity of human diabetes. It should be noted that two well-recognized experimental models of type 2 DM without associated obesity (i.e., partially pancreatectomized rats and rats subjected to a neonatal administration of streptozotocin) are characterized by a substantial reduction in beta-cell mass [24–26] that is considered to occur also in type 2 DM patients [27, 28]. In a commonly used genetic model of type 2 DM, the Goto-Kakizaki (GK) rat, beta -cell mass depletion precedes the onset of metabolic abnormalities [29]. In rats neonatally administered STZ (n-STZ rats), another remarkable difference from type 2 DM is the lack of insulin response to tolbutamide in the presence of glucose [30]. In GK rats, a stimulatory effect on insulin release from isolated islets has been shown after incubation with glucose and gliclazide [31]. In the present study, the experimental models of type 2 DM were modified from Masiello et al. [14]. The method took advantage of the partial protection exerted by suitable dosages of nicotinamide against the beta-cytotoxic effect of STZ to form an experimental diabetic syndrome in adult rats that appears closer to type 2 DM than other available animal models. Other advantages of this syndrome include 1) the facility of the induction of diabetes, 2) the high yield of

mildly diabetic animals, and 3) the stability of metabolic alterations. This is the reason this animal model was chosen in this study.

In our study, the RBO diet had higher SFA and MUFA contents but a lower PUFA content than did the soybean oil diet. In addition, the RBO diet contained 3.02 mg/g of α-tocopherol, 35.22 mg/g of γ-oryzanol, and 0.60 mg/g of γ-tocotrienol. The soybean oil was rich in α-tocopherol (0.45 mg/g) but did not contain γ-oryzanol or γ-tocotrienol. A study has shown an influence of RBO and γ-oryzanol activity on lipid metabolism in animals and humans [32]. In previous studies, nondiabetic rats were fed for 8 weeks either a cholesterol-containing or a cholesterol-free diet to which 10% RBO was added. This diet significantly decreased plasma TC, LDL-C, and VLDL-C levels [3, 33]. However, we did not observe a significant reduction in plasma TC, LDL-C, and TG in our study. This difference likely occurred because of the use of different animal models and different fat levels in the two studies.

In our study, the proportions of PUFAs and MUFAs in the plasma and the hepatic fatty acid compositions in the diabetic rats fed RBO were altered after the intake of RBO, which agrees with previous studies. Dietary fatty acids were previously shown to alter plasma and hepatic fatty acid compositions [34, 35]. In previous studies, it was shown that a diet with a high MUFA content decreased plasma TG, cholesterol, and LDL-C levels more than did a high-SFA diet [36]. However, we did not observe marked changes in cholesterol, LDL-C, and TG between these two groups. This comparison showed the effect of plasma and hepatic fatty acid composition on the blood lipid is multivariate. For example, the use of different animal models and different interventional periods will affect outcomes. We believe that the relationship between the plasma and hepatic fatty acid compositions and blood lipids needs further study.

In the present study, diabetic rats fed the RBO diet had a higher HDL-C concentration than did rats in the control group, which agrees with previous studies. For example, Sharma and Rukmini [37] showed that rats fed RBO, at the level of 10% in the diet, for 8 weeks had significantly lower plasma TC, LDL-C, and VLDL-C concentrations. They also observed a significant increase in plasma HDL-C. These findings showed that RBO significantly improve the plasma lipoprotein pattern in rats. In our study, diabetic rats fed the RBO diet had a lower TC/HDL-C ratio than did rats in the control group. Recent data from the Women's Health Study indicate that the use of non-HDL-C and the ratio of TC/HDL-C as predictors of CVD risk are superior to the use of TC and LDL alone [38]. Thus, we suppose that RBO could improve the plasma lipoprotein pattern and reduce the risk of CVD in T2DM rats.

The increased excretion of fecal neutral sterols and bile acids due to consumption of the RBO diet is explained in

part by the reduction in cholesterol reabsorption in the intestines. This finding agrees with previous studies in rats, which showed a mechanism of cholesterol absorption inhibition by phytosterols, the chemical structure of which is very similar to that of cholesterol. Phytosterols interfere with cholesterol movement into micelles and reduce cholesterol absorption in the intestines. In addition, phytosterols increase the excretion of bile acids, which results in a lowering of plasma and liver cholesterol levels [39]. In this study, we also found that RBO affected the fecal excretion of cholesterol and bile acids as well. It significantly increased the fecal excretion of bile acids and neutral sterols.

In the present study, none of the experimental diets contained cholesterol; thus, the neutral sterol in feces would have been catabolized mainly from cholesterol synthesized *in vivo*. We speculated that the HMG-CoA reductase activity–lowering effect of γ-tocotrienol in RBO was probably overshadowed by the effect of γ-oryzanol on HMG-CoA reductase activity, because γ-oryzanol consumption resulted in a higher excretion of fecal neutral sterols and bile acids, which resulted in increased cholesterol synthesis and catabolism *in vivo*.

Tabata and colleagues have shown that an intravenous injection of 0.5 to 4 mg/day phytosterol for 5 days resulted in a significantly increased conversion into bile acid in the rat [40]. Shefer and colleagues [41] reported in rats that sitosterol consumption at a level of 2% of the diet for 1 week increased the activity of cholesterol-7α hydroxylase, an enzyme that limits the rate of conversion of cholesterol into bile acids [42]. In our study, the excretion of bile acid and neutral sterols in feces increased significantly in T2DM rats fed an RBO diet rich in unsaponifiable compounds and γ-oryzanol. We surmised that the observed reduction of cholesterol in the liver may have been due to an increase in the activity of cholesterol-7α hydroxylase in terms of maintaining the homeostasis of bile acids.

The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [43]. Hypertriglyceridemia is also associated with the metabolic consequences of hyperinsulinemia, i.e., insulin resistance [44]. Levine and colleagues [45] used the product of the AUC for glucose and insulin during the oral glucose tolerance test to derive an estimate of insulin sensitivity. In our study, these variables improved in rats with STZ-induced T2DM fed an RBO diet ($p<0.05$). In addition, the rats with T2DM fed an RBO diet had a significantly lower AUC for insulin. This finding agrees with that of a previous study in which diabetic rats fed a 15% RBO diet had a significantly lower fasting insulin/glucose ratio than did those fed a soybean oil diet [46]. We found for the first time that diabetic rats fed an RBO diet exhibited a significantly suppressed hyperinsulinemic response to a high-fat diet. In a previous study, a diet high in MUFAs was shown to decrease postprandial plasma glucose and insulin levels [47].

In the present study, we speculated that rats fed the RBO diet had a suppressed hyperinsulinemic response that may have resulted from the higher MUFA dietary intake and lower hepatic TG concentrations than in rats fed the control diet. The reduction in hepatic TG accumulation is associated with decreased insulin resistance [48]. Insulin resistance is associated with an increase in the 3 main sources of TG for VLDL assembly: NEFA flux from adipose tissue to the liver, hepatic uptake of VLDL, intermediate-density lipoprotein, and chylomicron remnants, and de novo lipogenesis [49]. In T2DM, the ability of insulin to inhibit lipolysis is impaired and plasma NEFA concentrations are elevated [50]. Chronically elevated plasma NEFA concentrations aggravate insulin resistance [51]. However, the exact mechanism responsible for the suppression of the hyper-insulinemic response by RBO is not clear and requires further study. In addition, the results showed the body weight, weight gain, and epididymal fat weight had a lower tendency in RBO group than in control group. We observed that there was no significant differences of food intake between two groups, thus we speculate this tendency did not result from the taste problem of RBO but resulted from that RBO could affect the lipid metabolism via decreasing the AUC for insulin. When increasing in plasma insulin will stimulate the transportation of glucose into the adipocytes, as well as stimulate the synthesis of fat. In our study, we found that AUC for insulin decreasing significantly in RBO group and could lead to the reduction of lipogenesis. However, we think a longer term study is needed to clarify the mechanism.

In conclusion, consumption of the RBO diet resulted in a significant reduction in the AUC for insulin, the plasma NEFA concentration, hepatic cholesterol, and the TC/HDL-C ratio and an increase in plasma HDL-C and neutral sterol and bile acid excretion. We speculate that RBO improves insulin resistance and lipid metabolism in rats with T2DM.

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