Dietary Supplementation with Raspberry Seed Oil Modulates Liver Functions, Inflammatory State, and Lipid Metabolism in Rats

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Abstract

Background: Although raspberry seed oil (RO) is rich in essential fatty acids there is a lack of experiments assessing benefits of its consumption.

Objective: We investigated the effects of dietary supplementation with RO on healthy rats and rats with low-grade systemic inflammation, liver disorders, and dyslipidemia induced by a high-fat/low-fiber (HF/LF) diet.

Methods: Thirty-two rats were allocated into 4 groups of 8 rats each and fed for 8 wk with a control (C; 7% lard and 5% cellulose) or HF/LF (21% lard and 2% cellulose) diet or modifications of these diets in which 7% RO replaced all (C+RO group) or a proportion of (HF/LF+RO group) the lard. Effects of diet and RO and their interaction on bacterial activity and metabolite formations in the distal intestine, liver fat and glutathione concentration, plasma lipid profile, transaminase activities, and plasma concentrations of C-reactive protein (CRP) and tumor necrosis factor alpha (TNF-α) were tested.

Results: Dietary RO decreased plasma alanine and aspartate transaminase activities (43.4 and 157 vs. 25.6 and 115 U/L, respectively; P < 0.05 and P < 0.005) and plasma TNF-α and triglyceride concentrations (132 pg/mL and 2.07 mmol/L vs. 86.5 pg/mL and 0.99 mmol/L, respectively; P < 0.05). In C+RO group livers, the fat concentration was decreased, whereas the glutathione to glutathione disulfide ratio was increased compared with the C group (30.1% and 6.20 μmol/g vs. 23.3% and 7.25 μmol/g, respectively; P ≤ 0.05); however, those differences were not observed between the HF/LF groups (Pinteraction < 0.05). In the HF/LF+RO group, the plasma CRP concentration was lower than in the HF/LF group (88.1 vs. 765 pg/mL; P ≤ 0.05) and similar to that in the C and C+RO groups (158 and 128 pg/mL, respectively).

Conclusion: Dietary RO improves plasma lipid profile and liver functions and reduces low-grade systemic inflammation in rats; however, the extent of these beneficial effects is partly dependent on the diet type. J Nutr doi: 10.3945/jn.115.212407.

Keywords: essential fatty acids, cecal microbiota, liver functions, triglycerides, transaminases, Wistar rats

Introduction

The consumption of fruits has been associated with a decreased risk of the development of many chronic diseases, such as obesity and cardiovascular disease (1). Raspberries (Rubus idaeus L.) are popularly consumed fruits both fresh and as an ingredient of processed food products. These fruits are a rich source of nutrients and bioactive compounds that may benefitably affect human health (2). To date, most raspberry-related studies have focused on the health effects of its juice and seed extracts (3–5). During raspberry processing, however, many valuable compounds are wasted. For example, pomace and seeds are usually considered waste, but these components may be useful for nutritional purposes (6, 7). Raspberry seeds contain ~23% of oil that is abundant in essential FAs and that has a favorable ratio of n–6 to n–3 FAs (2–3 to 1) (6, 8). Raspberry seed oil (RO) is also a source of antioxidant vitamins and bioactive compounds, such as tocopherols, carotenoids, and polyphenols (3, 8, 9). Notably, a recent toxicologic study in Wistar rats showed some antioxidant activity of RO (10). Nevertheless, there is still a lack of nutritional experiments to assess potential benefits from the incorporation of RO into diets.

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3 Supplemental Table 1 is available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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Abbreviations used: ACL, the antioxidant capacity of lipid-soluble substances; ACW, antioxidant capacity of water-soluble substances; AL, atherogenic index; ALT, alanine transaminase; AST, aspartate transaminase; C, control; CRP, C-reactive protein; D, type of diet; GSH, reduced glutathione; GSSG, oxidized glutathione; HF/LF, high-fat/low-fiber; RO, raspberry seed oil; TC, total cholesterol; α-gal, α-galactosidase; α-gluc, α-glucosidase; β-gal, β-galactosidase; β-gluc, β-glucosidase.
A diet rich in fats, especially SFAs, and low in dietary fiber increases the risk of obesity, type 2 diabetes, and cardiovascular disease (11, 12). An insufficient amount of dietary fiber in the diet can adversely affect the gastrointestinal tract functions and change the microbiota of the distal intestine, which is thought to contribute to the development of the aforementioned diseases and colorectal cancer (13). Thus, the consumption of dietary fiber as a part of whole-grain foods, vegetables, and fruits is widely recommended (14). Replacing dietary saturated fats with unsaturated fats derived from vegetable oils is also recommended, especially because of the presence of PUFAs in the oils, which are thought to play an important role in the prevention of cardiovascular disease. The beneficial effects of PUFAs are reflected in the improvement in blood lipid profile, blood pressure, and the immune response of the organism (15–17). However, there are still some controversies about the bioactivity of PUFAs. For example, a dietary overabundance of long-chain n-3 PUFAs may trigger oxidative stress, whereas the overconsumption of linoleic acid (18:2n-6) may promote inflammation (18, 19). In addition, oils are usually a mixture of several PUFAs, thereby making it difficult to predict the outcome of their consumption.

The aim of this study was to examine the effects of dietary supplementation with RO on the intestinal and liver functions, inflammatory response, antioxidant status, and lipid metabolism of healthy rats and rats with disorders induced by a high-fat/low-fiber (HF/LF) diet. The hypothesis is that the biological activity of dietary RO can be beneficial for both groups of rats, but to a different extent.

Methods

**RO and its chemical analysis.** Unrefined cold-pressed raspberry (Rubus idaeus L.) seed oil was purchased from Greenaction. The FA profile of the oil was determined after the conversion of the FAs into methyl esters by using a GC method previously described (20). The FA profile of the oil is shown in Table 1.

**Animal study.** The animal protocol used in the present study was approved by the Local Institutional Animal Care and Use Committee (Olsztyn, Poland). The nutritional experiment was performed on 32 male Wistar rats allocated to 4 groups of 8 animals each that were housed individually in plastic cages. The initial body weight was comparable among groups and equaled 128 ± 5.2 g, on average. For 8 wk, each group was fed a modified version of the semipurified rodent diet recommended by Reeves (21) (details shown in Supplemental Table 1). The control group (C group) was fed a control diet that contained 8% fat (including 7% lard and 1% canola) and 5% cellulose as the source of fiber. The HF/LF group was fed a diet that contained 22% fat (including 21% lard and 1% canola), 0.5% cholesterol, and 2% cellulose. The C+RO and HF/LF+RO groups were fed the control and HF/LF diets, respectively, containing 7% RO, which was added in place of a proportion of lard. Details about the proportional composition of each group-specific diet are shown in Supplemental Table 1. The diets were freshly prepared at weekly intervals, stored in hermetic containers at −20°C, and administered ad libitum. The individual body weights of rats and food intakes were recorded on a weekly and daily basis, respectively. The rats were maintained under standard conditions at a temperature of 21–22°C and a relative air humidity of 50–70%, with intensive room ventilation (15 times/h) and a 12-h lighting regimen.

**Sample collection and analysis.** At the termination of the experiment, the rats were anesthetized with sodium pentobarbital according to the recommendations for killing of laboratory animals (50 mg/kg body weight). After laparotomy, blood samples were collected from the caudal vein and stored in tubes containing EDTA. The cecum, colon, and liver were removed and weighed. Immediately after being killed (−10 min), cecal and colonic pH values were measured (model 301; Hanna Instruments). Fresh cecal digesta were used to determine the ammonia concentration, which was extracted, trapped in a solution of boric acid, and quantified by direct titration with sulfuric acid (22). SCFAs were measured by using GC. A known amount of fresh cecal digesta was mixed with 0.2 mL formic acid and stored at −80°C. Afterward, the sample was diluted with deionized water, centrifuged at 10,000 × g for 5 min at 4°C, and filtered through a 0.45-μm membrane. The supernatant was then decanted for injection into a gas chromatograph (Shimadzu GC-14A; Shimadzu Corporation) and analyzed by using conditions described elsewhere (20). The cecal and colonic activity of selected bacterial enzymes (α- and β-glucosidase as well as α- and β-galactosidases) was measured by the rate of p- or o-nitrophenol release from their nitrophenyl glucosides according to a previously described method (23).

The liver fat mass was determined shortly after dissection by time-domain NMR (Minispec LF 90II; Bruker). After storage of the liver at −20°C, the reduced glutathione (GSH) and oxidized glutathione (GSSG) concentrations were determined by using an enzymatic recycling method described by Rahman et al. (24).

The blood was centrifuged for 15 min at 380 × g at 4°C, and the obtained plasma was then stored at −20°C until analysis. Plasma TG, total-cholesterol (TC), and HDL-cholesterol concentrations and the plasma activity of aspartate transaminase (AST) and alanine transaminase (ALT) were estimated by using reagents from Alpha Diagnostics Ltd. On the basis of the plasma lipid profile, the atherogenic index (AI) was calculated as previously described by using the following formula: log[TGs (mmol/L)/HDL cholesterol (mmol/L)] (25). The plasma concentration of C-reactive protein (CRP) and TNF-α were determined by using validated rat enzyme immunoassay kits (Rat C-Reactive Protein ELISA and Cusabio and Rat TNF alpha ELISA kits, respectively; Thermo Scientific). The plasma antioxidant capacity of water-soluble substances (ACW) and the plasma antioxidant capacity of lipid-soluble substances (ACL) were determined by a photochemiluminescence detection method with the use of a Photochem analyzer (Analytik Jena) and respective kits (ACW and ACL kits; Analytik Jena). In the photochemiluminescence assay, the generation of free radicals was partially eliminated through reactions with antioxidants present in the plasma samples, and the remaining radicals were quantified by luminescence generation. Ascorbate and Trolox calibration curves were used to evaluate ACW and ACL, respectively.

**Statistical analysis.** Statistica software (version 8.0; StatSoft Corporation) was used to determine whether variables differed between the treatment groups. Two-factor ANOVA was applied to assess the effects of diet type (D1 control or HF/LF), the RO addition, and P-interaction between these investigated factors (D × RO). If ANOVA revealed a significant P-interaction (P ≤ 0.05), the differences between the individual groups were then assessed with Duncan’s multiple range post hoc test at P ≤ 0.05. If the variance was unequal, the Kruskal-Wallis 1-factor ANOVA by ranks was used followed by Dunn’s post hoc test. The results are presented as means ± SEMs, except for the FA contents of RO, which are expressed as means ± SDs.

**Results**

After the 8-wk feeding period, the dietary intake was significantly lower with the HF/LF diet than with the control diet...
was affected by both dietary factors (D and RO; P < 0.05). Nevertheless, caloric intake and body weight gain were significantly greater with the HF/LF diet than with the control diet (67.3 ± 0.99 vs. 61.6 ± 1.12 kcal/d and 246 ± 6.71 vs. 225 ± 7.42 g, respectively; P < 0.001 and P < 0.05). Dietary RO did not affect caloric intake nor the growth of the rats.

Basic indices of the hindgut and SCFA concentrations in the cecal digesta are shown in Table 2. The relative cecal tissue and digesta mass were not affected by the tested factors (P > 0.05). The colon tissue mass was significantly less in the HF/LF+RO group than in the HF/LF group, whereas in the C+RO group the mass was not different than in the C group (P-interaction < 0.05). The colon digesta mass was reduced by the HF/LF diet compared with the control diet (P < 0.05). The effect of the tested factors on the propionate concentration in the cecal digesta was not clear (P-interaction < 0.05). In the C group, the propionate concentration was significantly higher than that in the C+RO group, whereas in the HF/LF group, the propionate concentration was significantly lower than that in the HF/LF + RO group (P ≤ 0.05). The butyrate concentration in the cecal digesta was decreased by the HF/LF diet (P < 0.05). The activity of microbial enzymes in the hindgut digesta is shown in Table 3. Cecal β-glucosidase activity was significantly lower in both HF/LF groups compared with the C+RO group. Cecal α-galactosidase activity was decreased by the HF/LF diet (P < 0.05) and was increased by the addition of RO (P < 0.01). In the colonic digesta, β-glucosidase activity was increased by dietary RO (P < 0.01), whereas α-galactosidase activity was not affected by the diet or by the oil (both P = 0.06).

The HF/LF diet increased the relative liver mass and the proportion of fat in the liver (P ≤ 0.01 and P ≤ 0.01, respectively; Table 4). In addition, a significant P-interaction effect between the type of diet and the presence of RO in the diet was observed on the proportion of fat in the liver (P < 0.05), resulting in its lesser value in the C+RO group than in the C group (P ≤ 0.05). However, the liver fat proportion was highest in both HF/LF groups (P ≤ 0.05). The glutathione status in the rat livers is shown in Figure 1. In rats fed the HF/LF diet, the liver GSH concentration was decreased, whereas the liver GSSG concentration was increased compared with the control diet (P < 0.05 and P < 0.01, respectively). The liver GSH:GSSG ratio was affected by both dietary factors (D and RO; P < 0.001 and P < 0.05, respectively), and a P-interaction effect was also observed (D × RO; P < 0.01). Hence, the GSH:GSSG ratio was higher in the C+RO group than in the C group, but the lowest ratio was found in both HF/LF groups (P ≤ 0.05). The plasma ALT activity was elevated by the HF/LF diet and decreased by the addition of RO (P < 0.01 and P < 0.05, respectively). Dietary RO also decreased plasma AST activity (P < 0.01).

Plasma TNF-α concentration was elevated by the HF/LF diet and decreased by dietary RO (P < 0.01 and P < 0.05, respectively). Moreover, a P-interaction effect between the type of diet and the presence of RO was observed on the plasma CRP concentration, resulting in its higher concentration in the HF/LF group than in the other groups (P < 0.05).

The plasma lipid profile was affected by both tested factors and is shown in Table 5. TC and non-HDL-cholesterol concentrations were increased (P < 0.001), whereas the HDL-cholesterol concentration was decreased (P < 0.05) by the HF/LF dietary regimen. The RO decreased the TG concentration and the AI of plasma (P < 0.05 and P < 0.01, respectively), whereas the non-HDL-cholesterol concentration was not significantly affected by this dietary factor (P = 0.07).

### Discussion

The RO used in this study was especially rich in linoleic and α-linolenic acid (18:3n–3; 50.7% and 29.2%, respectively), which is similar to the results obtained by other authors who determined the FA profile of RO (6, 10, 26). However, in the aforementioned studies, the contents of linoleic and α-linolenic acid were higher by up to 4% and 5.5%, respectively, which can be a consequence of the different growth conditions of raspberries or the extraction method that was applied.

Dietary fiber is, in part, an energetic substrate for intestinal microbiota, which can metabolize it to SCFAs. However, recent findings also indicated that the type and amount of dietary fat can also be an important factor affecting the intestinal microbiota. de Wit et al. (27) showed that a diet high in saturated fat reduces microbial diversity in mice, which may be due to an overflow of dietary fat into the distal parts of the intestine. Moreover, we previously showed that both the amount and type of dietary fat can significantly change microbial glycolytic activity and metabolite formation in the distal intestine, but the

### Table 2: Tissue and digesta mass, pH, and SCFA concentrations in the hindgut of rats fed a control or an HF/LF diet without or with RO for 8 wk

<table>
<thead>
<tr>
<th>Group</th>
<th>C</th>
<th>C+RO</th>
<th>HF/LF</th>
<th>HF/LF+RO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>D</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Digesta mass, g/100 g BW</td>
<td>3.45 ± 0.25</td>
<td>3.20 ± 0.24</td>
<td>3.10 ± 0.31</td>
<td>3.13 ± 0.35</td>
</tr>
<tr>
<td>pH</td>
<td>6.95 ± 0.13</td>
<td>7.27 ± 0.17</td>
<td>7.41 ± 0.06</td>
<td>7.25 ± 0.07</td>
</tr>
<tr>
<td>SCFA Concentrations, µmol/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>121 ± 7.56</td>
<td>105 ± 4.59</td>
<td>90 ± 2.92</td>
<td>85 ± 4.19</td>
</tr>
<tr>
<td>Propionate</td>
<td>196 ± 12.44</td>
<td>18.3 ± 0.92</td>
<td>14.8 ± 0.88</td>
<td>18.4 ± 0.94</td>
</tr>
<tr>
<td>Butyrate</td>
<td>23.7 ± 2.40</td>
<td>22.8 ± 1.87</td>
<td>11.6 ± 2.19</td>
<td>10.6 ± 1.61</td>
</tr>
<tr>
<td>Total</td>
<td>174 ± 10.54</td>
<td>150 ± 6.21</td>
<td>123 ± 4.49</td>
<td>121 ± 4.06</td>
</tr>
</tbody>
</table>

1. Values are means ± SEMs, n = 8. Means within a column without a common letter differ, P ≤ 0.05 (Duncan’s post hoc test). NS, P > 0.05. BW, body weight; C, control; D, type of diet; HF/LF, high-fat/low-fiber; RO, raspberry seed oil.
2. C and HF/LF groups were fed a control and an HF/LF diet, respectively; C+RO and HF/LF+RO groups were fed a control and an HF/LF diet containing 7% RO, respectively.
3. Analyzed by using Kruskal-Wallis test.

Dietary supplementation with raspberry seed oil

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It is worth mentioning that microbial groups compared with the C+RO group, whereas colonic obtained after the addition of RO to the diets. In addition, cecal galactosidase activity, whereas a completely opposite effect was ambiguous effects on the organism. The activity of linked to some extent to the amount of cellulose in the HF/LF observed changes in the microbial activity should also be directly metabolites, which are assumed to be beneficial for the body, mycotoxins, such as deoxynivalenol, and for the release of in the distal intestine is responsible both for the generation of 4 of 7 Fotschki et al. changes might be associated with the increased secretion of bile agents both alone and in conjunction with diet-derived FAs acids into the small intestine because they are potent antimicroorganisms (29–31). In the present study, the HF/LF diet lowered bial agents both alone and in conjunction with diet-derived FAs antimicrobials (20, 28). Nevertheless, these changes might be associated with the increased secretion of bile acids into the small intestine because they are potent antimicrobials both alone and in conjunction with diet-derived FAs (29–31). In the present study, the HF/LF diet lowered antimicrobials by-product, which is an indigestible food component that can increase observed changes in the microbial activity should also be directly linked to some extent to the amount of cellulose in the HF/LF diet, which is an indigestible food component that can increase the bulk of digesta and the fecal output (13, 34). Indeed, in this study, the HF/LF diet decreased the colonic digesta mass compared with the control diet. Furthermore, changes in the microbial activity were partly consistent with the cecal production of SCFAs observed in the present study. The cecal butyrate concentration was significantly lower with the HF/LF diet than with the control diet, which was accompanied by increased acidity of the cecal digesta. However, the addition of the RO to the HF/LF diet significantly increased the propionate concentration in the cecum.

In this study, changes in cecal propionate production were consistent with those observed in the blood lipid profile. It has been suggested that propionate is involved in the cholesterol-lowering effect of dietary fiber by impairing acetate utilization, especially when cholesterol synthesis is activated to compensate for enhanced falcinal losses of steroids (35). Indeed, the HF/LF diet decreased propionate production, which was associated with the increased TC and non–HDL-cholesterol concentrations as well as with the decreased HDL-cholesterol concentration. Moreover, the plasma TG concentration and the plasma AI were significantly decreased after dietary supplementation with the PUFA-rich RO. The TG-lowering activity of PUFA is well documented in the literature and is mediated by the inhibition of hepatic lipase activity and liver VLDL synthesis as well as by the enhancement of hepatic lipoprotein lipase activity (36–38). The observed TG-lowering effect of the dietary RO was as efficient as that of some lipid-lowering drugs (39). Similar TG-lowering

| TABLE 4 | Liver mass and fat content, plasma transaminase activities, CRP and TNF-α concentrations, and antioxidant status in rats fed a control or an HF/LF diet without or with RO for 8 wk1 |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Group2                  | Liver                    | Plasma                   |
|                         | Mass, g/100 g BW | Fat, % | ALT, U/L | AST, U/L | CRP, pg/mL | TNF-α, pg/mL | ACL, mmol/L | ACW, mmol/L |
| C                       | 3.32 ± 0.10          | 30.1± 1.75           | 30.7 ± 1.81 | 138 ± 7.36 | 158± 44.3 | 95.6 ± 22.7 | 19.2 ± 1.03 | 2.80 ± 0.299  |
| C+RO                    | 3.28 ± 0.06          | 23.3± 0.79           | 26.2 ± 2.02 | 109 ± 5.62 | 128± 68.4 | 67.7 ± 12.3 | 20.6 ± 1.22 | 2.76 ± 0.202  |
| HF/LF                   | 4.75 ± 0.13          | 39.8± 2.72           | 56.0 ± 4.56 | 176 ± 10.9 | 765± 95.6 | 164 ± 16.7 | 21.8 ± 0.80 | 4.14 ± 1.310  |
| HF/LF+RO                | 4.14 ± 0.09          | 42.9± 1.48           | 28.6 ± 1.86 | 121 ± 2.20 | 88.1± 33.2 | 103 ± 22.0 | 22.2 ± 1.19 | 2.67 ± 0.319  |
| P (2-factor ANOVA)      | <0.001                | <0.001               | <0.01      | NS        | <0.05     | NS        | NS        | NS          |
| D × RO                  | NS                    | <0.05                | NS        | <0.05     | NS        | NS        | NS        | NS          |

1 Values are means ± SEMs, n = 7. Means within a column without a common letter differ, P ≤ 0.05 (Dunnett’s post hoc test). NS, P > 0.05. ACL, antioxidant capacity of lipid-soluble substances; ACW, antioxidant capacity of water-soluble substances; ALT, alanine transaminase; AST, aspartate transaminase; BW, body weight; C, control; CRP, C-reactive protein; D, type of diet; HF/LF, high-fat/low-fiber; RO, raspberry seed oil.

2 C and HF/LF groups were fed a control and an HF/LF diet, respectively; C+RO and HF/LF+RO groups were fed a control and an HF/LF diet containing 7% RO, respectively.

3 Analyzed by using Kruskal-Wallis test.

4 Values are means ± SEMs, n = 8. Means within a column without a common letter differ, P ≤ 0.05 (Dunnett’s post hoc test). NS, P > 0.05. C, control; D, type of diet; HF/LF, high-fat/low-fiber; RO, raspberry seed oil; a, b, c, d analyzed by using Kruskal-Wallis test. Means within a row without a common letter differ, P < 0.05. D, high-fat/low-fiber; RO, raspberry seed oil; NS, P > 0.05. a, b, c, d analyzed by using Kruskal-Wallis test. Means within a row without a common letter differ, P < 0.05.

5 Values are means ± SEMs, n = 8. Means within a column without a common letter differ, P ≤ 0.05 (Dunnett’s post hoc test). NS, P > 0.05. C, control; D, type of diet; HF/LF, high-fat/low-fiber; RO, raspberry seed oil; a, b, c, d analyzed by using Kruskal-Wallis test. Means within a row without a common letter differ, P < 0.05. D, high-fat/low-fiber; RO, raspberry seed oil; NS, P > 0.05. a, b, c, d analyzed by using Kruskal-Wallis test. Means within a row without a common letter differ, P < 0.05.
effects were also observed by Ash et al. (40) who added black raspberry seed oil to the diet of Syrian hamsters. However, Pieszka et al. (10) did not find any significant effect of RO on the blood lipid profile of rats, which may have been due to a lack of equal caloric intake between groups because it was an oral toxicity test.

The HF/LF diet used in this study induced typical symptoms of fatty liver disease, including increased hepatic fat accumulation and plasma ALT activity as well as a decreased GSH:GSSH ratio, which suggests serious disturbances in the oxidative balance of the liver (40). Moreover, the HF/LF diet slightly but significantly increased the plasma CRP concentration, indicating a low-grade systemic inflammation in the organism, which can be due to endotoxins derived from intestinal bacteria (41). Cani et al. (42) noted some changes in the hindgut microbiota, higher intestinal permeability, and metabolic endotoxemia as a consequence of a high-fat diet in mice. Because the cecal butyrate concentration responsible for the maintenance of the intestinal barrier was 2 times lower after the HF/LF diet, infiltration of bacterial endotoxins to the bloodstream may have occurred in the present study as well (41, 43). In contrast, PUFAs can decrease the production of TNF-\(\alpha\), which is especially important because increased plasma concentrations of TNF-\(\alpha\) stimulate the release of CRP from the liver and adipose tissues (44). In our study, RO supplementation decreased the plasma TNF-\(\alpha\) concentration and prevented the increase in plasma CRP concentration in rats fed the HF/LF diet. Moreover, dietary RO benefically decreased plasma ALT and AST activities as well as reduced hepatic fat concentration, but this effect was visible only for the control group. The n–6 to n–3 ratio of the examined oil was 1.7:1 and similar ratios have been shown to be beneficial for the treatment of liver inflammation and for reduction in hepatic fat accumulation (45, 46). In a study of rats with induced hepatic steatosis, Marsman et al. (47) showed that n–3 PUFAs reduced both serum ALT activity and hepatic fat concentration. In the present study, the disturbances caused by the components of the HF/LF diet, such as the relatively higher content of SFAs, were apparently too considerable to be fully overcome by the tested oil. Furthermore, n–3 PUFAs can act as antioxidants in vascular endothelial cells, which can be beneficial for cardiovascular health (48). In contrast, high doses of n–3 PUFAs may trigger oxidative stress, mostly due to their high degree of unsaturation, which makes them potential substrates for the formation of lipoperoxides (18). In a study of diabetic rats fed a high-fat diet, however, de Assis et al. (49) showed some beneficial effects of n–3 PUFAs on oxidative stress induced in the liver, including a

**TABLE 5** Plasma lipid profile and AI in rats fed a control or an HF/LF diet without or with RO for 8 wk\(^1\)

| Group\(^2\) | TGs, mmol/L | TC, mmol/L | HDL-C, mmol/L | Non-HDL-C, mmol/L | AI | RO
|------------|------------|------------|---------------|-------------------|-----|-----
| C         | 2.62 ± 0.23| 2.00 ± 0.08| 1.26 ± 0.09   | 0.74 ± 0.05       | 0.31 ± 0.05 |
| C+RO      | 1.17 ± 0.17| 1.96 ± 0.08| 1.42 ± 0.05   | 0.54 ± 0.05       | 0.11 ± 0.07 |
| HF/LF     | 1.52 ± 0.21| 3.13 ± 0.12| 0.95 ± 0.04   | 2.18 ± 0.12       | 0.19 ± 0.06 |
| HF/LF+RO  | 0.81 ± 0.07| 2.15 ± 0.16| 0.84 ± 0.09   | 1.32 ± 0.19       | 0.01 ± 0.06 |

\(^1\) Values are means ± SEMs, \(n = 7\). NS, \(P > 0.05\). AI, atherogenic index, \(\log [\text{TG (mmol/L)/HDL-C (mmol/L)}]\); C, control; D, type of diet; HDL-C, HDL cholesterol; HF/LF, high-fat/low-fiber; Non-HDL-C, the difference between TC and HDL-C; RO, raspberry seed oil; TC, total cholesterol.

\(^2\) C and HF/LF groups were fed a control and an HF/LF diet, respectively; C+RO and HF/LF+RO groups were fed a control and an HF/LF diet containing 7% RO, respectively.
reduction in lipoperoxide concentrations and superoxide dismutase to catalase enzymatic ratios as well as an increase in manganese superoxide dismutase concentrations. In addition, Pieszka et al. (10) indicated that the administration of RO to rats by gavage for 5 wk led to a reduced cellular GSSG activity of erythrocytes. This is in accordance with the present study, in which the liver GSH:GSSG ratio was partly increased by dietary RO. However, the observed antioxidative effects of dietary RO may also be associated with other bioactive compounds that are present in the oil, such as tocopherols and carotenoids (3, 8, 9).

In conclusion, the HF/LF diet used in this study led to a series of metabolic disorders in the distal intestine, liver, and blood plasma of rats. In contrast, the consumption of RO improved lipid metabolism and reduced low-grade systemic inflammation in rats. The addition of RO can also improve liver functions by lowering plasma transaminase activities and liver fat mass as well as by increasing the antioxidant status of the liver, but the extent of these beneficial effects may be less when the oil is supplemented with an unbalanced diet. Moreover, dietary RO also has some equivocal effects on the microbial metabolism in the distal intestine. Nevertheless, the present study showed that RO can be a valuable source of essential FAs in the daily diet.

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BF and AJ designed the research and wrote the manuscript; BF, AJ, and JJ conducted the research; AJ analyzed the data; and AJ and ZZ had primary responsibility for the final content. All authors read and approved the final manuscript.

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6 of 7 Fotschkı et al.