Journal of Animal and Feed Sciences, 22, 2013, 137–143
The Kielanowski Institute of Animal Physiology and Nutrition, PAS, Jabłonna

Resveratrol affects the lipid profile but not antioxidant enzymes gene expression in rats fed hypercholesterolaemic diet

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KEY WORDS: resveratrol, high-fat diet, lipid profile, antioxidant enzymes, gene expression, rats

Received: 6 November 2012
Revised: 27 February 2013
Accepted: 17 June 2013

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ABSTRACT. The objective of this study was to evaluate the effect of adding resveratrol (RSV) to a hypercholesterolaemic diet on the lipid profile, activity of antioxidant enzymes and their mRNA gene expression, as well as lipid concentration in selected organs of Wistar rats. Animals were divided into four groups and fed experimental diets for 8 weeks. The negative control group (NC) was fed AIN-93G diet. The positive control group (PC) received a hypercholesterolaemic diet (AIN-93G + 0.1% cholesterol, 7% butter). The other groups were fed PC diets supplemented with 0.05% and 0.1% RSV, respectively. An addition to the PC diet RSV (0.05 and 0.1%, respectively) significantly decreased concentration of total cholesterol (TC) and LDL-cholesterol in serum of rats compared to the PC group. The triacylglycerols (TAG) level was significantly lower in serum of rats fed with 0.05% of RSV in comparison to other experimental groups. Activities of antioxidant enzymes and their mRNA gene expression were not affected by RSV. In conclusion, relatively high doses of RSV were sufficient in lowering TC and LDL-cholesterol, and TAG and oxidative stress without involving naturally occurring mechanisms in rats.

Introduction

Resveratrol (3,4',5-trihydroksystilbene) (RSV) belongs to non-nutrient bioactive compounds. Its healthy properties are connected with antioxidant, anti-inflammatory and anti-aging properties. In several studies it was reported that RSV decreases oxidative stress in various animal models and may be used in the prevention and treatment of non-communicable diseases, e.g. dyslipidaemia, obesity, hypertension and diabetes (Lagouge et al., 2006; Leiro et al., 2010; Kim et al., 2011).

An unbalanced diet, especially a high intake of saturated fatty acids and cholesterol, is a risk factor for many diseases: hypertension, obesity and cardiovascular diseases (Kaput, 2004; Cho et al., 2012). It is causes by an increased level of LDL-cholesterol (Artaud-Wild et al., 1993, Yamagishi et al., 2013). Additionally it has been also well reported that cholesterol in the presence of animal origin fat (butter or lard) added to the experimental diets increases total cholesterol (TC), LDL-cholesterol and oxidative stress (mainly by oxidative modification of LDL lipoproteins) in animal models (Yuan et al., 1998; Pisulewski et al., 2002; Yuan and Kitts, 2003; Rocha et al., 2009; Zawistowski et al., 2009).

Reactive oxygen species (ROS) in the body can cause lipid and protein oxidation, DNA damage, modification and modulation of gene expression. Imbalance between ROS and antioxidants causes oxidative stress (Kim et al., 2011; Kitada et al., 2011). In the human body several mechanisms are involved in the scavenging of ROS for example antioxidant enzymes such as: glutathione reductase (GSR), super-

oxide dismutase (SOD), haeme oxygenase 1 (HO-1). The human body usually requires more substances which will neutralize ROS. Naturally occurring in daily diets, antioxidants and other chemical bioactive compounds help detoxify ROS and prevent the damaging of cellular macromolecules and organelles through several metabolic pathways. Several studies with different animal models reported that the addition of RSV in dose-depended manner to various experimental diets (with high-fat content, e.g. 52% soya bean oil, 42% lipids or 60% energy from fat; or addition of fructose to diets in high amount, e.g. 63%) increases the production of antioxidant enzymes in tissues and decreases the content of ROS (Aubin et al., 2008; Rocha et al., 2009; Kitada et al., 2011; Tauriainen et al., 2011; Kopeć et al., 2013).

In this research it was hypothesized that the addition of cholesterol and butter to the experimental diets increase lipid profile and oxidative stress, but the addition of resveratrol decreases them, without involving naturally occurring mechanisms.

The objective of this study was to evaluate the effect of the addition of RSV to the hypercholesterolaemic diet on lipid profile, the activity of antioxidant enzymes and their mRNA gene expression, as well as lipid concentration in selected organs of Wistar rats.

Material and methods

Animal study

Five weeks-old male Wistar rats (n=24), were purchased from Animal Husbandry in Warsaw (Poland). All experimental procedures were approved by I Regional Ethics Committee on Animal Experimentation in Krakow (Poland). Before the experiment animals were acclimatized for 7 days on standard laboratory diet. At the beginning of the experiment the average body mass of animals was 116±10 g. After the adaptation period rats were randomly divided into four groups (n=6) and fed with diets prepared based on AIN-93G (Reveers, 1997). The composition of experimental diets is shown in Table 1. The animals were housed separately in stainless steel metabolic cages at 25°C and 12/12 h – light/dark cycle. During the experiment, animals had free access to water and diets. Intakes of diets were recorded every day. Body weight gain was recorded during the whole experiment every week.

At the end of the experiment (after 8 weeks), fasted rats were anaesthetized with thiopental (Biocheme GmbH, Austria). Blood was obtained by heart puncture. Parts of blood samples were collected in heparinized tubes. Other parts of blood samples were collected to obtain serum by centrifugation (1500 *g*, 15 min). Livers, kidneys and hearts were dissected, washed in 0.9% sodium chloride, dried with laboratory tissue paper and weighed. Serum and tissue samples were kept frozen in –80°C until the analysis.

Table 1. Composition of experimental diets, g · kg^{-1} diet

Ingredient	NC	PC	PC + RSV 0.05%	PC + RSV 0.1%
Maize starch	532.486	522.486	521.986	521.486
Caseine	200	200	200	200
Saccharose	100	100	100	100
Soya bean oil	70	–	–	–
Butter	–	70	70	70
Fibre	50	50	50	50
Mineral mix[1]	35	35	35	35
Vitamin mix[1]	10	10	10	10
Choline	2.5	2.5	2.5	2.5
TBHQ[2]	0.014	0.014	0.014	0.014
Cholesterol	–	10	10	10
Resveratrol	–	–	0.5	1
Gross energy, kcal	3960	3920	3918	3916
Fatty acids composition, %				
4:0	–	3.25	3.10	3.10
6:0	–	2.29	2.25	2.21
8:0	–	1.40	1.40	1.34
10:0	0.056	3.14	3.13	3.41
12:0	0.067	3.44	3.43	3.50
14:0	0.227	11.35	11.38	11.85
14:1	0.011	1.01	1.00	1.04
15:0	0.030	1.00	1.01	1.06
16:0	10.12	34.30	34.20	34.59
16:1 (n-9)	0.022	0.10	0.11	0.10
16:1 (n-7)	0.087	1.56	1.60	1.61
17:0	0.073	0.46	0.48	0.47
17:1	0.039	0.23	0.21	0.21
18:0	3.96	9.01	9.07	9.20
18:1 (n-9)	23.15	21.56	21.61	21.58
18:1 (n-7)	2.25	1.96	2.00	1.92
18:2 (n-6)	53.90	2.54	2.51	2.41
18:3 (n-6)	–	0.07	0.07	0.07
18:3 (n-3)	5.61	0.43	0.38	0.38
20:0	0.27	0.096	0.10	0.10
20:1	0.14	0.07	0.07	0.07

NC-AIN-93G diet; PC – hypercholesterolaemic diet; PC+0.05% RSV –- hypercholesterolaemic diet with addition of 0.05% RSV; PC+0.1% RSV – hypercholesterolaemic diet with addition of 0.1% RSV, [1]AIN-93G; TBHQ[2] – tert-butylohydroquinone

Composition of fatty acids in experimental diets

The composition of fatty acids of the experimental diets was analysed by gas chromatography after the extraction of lipids from the experimental diets using the Folch's method (Folch et al., 1957). Fatty acids were esterified with 0.0025 M sodium

methoxide (de Man, 1964) and separated with the Trace GC Ultra model (Thermo Scientific Electron Corporation, USA), using a capillary Supelcowax 10 column (30 m × 0.25 mm × 0.25 μm; Supelco, Bellefonte, PA, USA). The carrier gas was helium with the flow rate of 5 ml · min^{-1}. The temperature of column was kept for 3 min at 60°C then increased up to 200°C (7°C · min^{-1}). This temperature was held for 20 min. The temperature of detector was 250°C, and split flow was 10 ml · min^{-1} (de Man, 1964; Domagała et al., 2010).

Serum and blood analysis

Serum was analysed for the concentration of total cholesterol (cat no. Liquick Cor-CHOL60 2-204), HDL-cholesterol (cat no. Cormay HDL 2-052) and triacylglycerides (cat no. Liquick Cor-TG60 2-253) (PZ Cormay S.A. Lublin, Poland). The differences between TC and HDL were used for calculations of LDL level (Friedewald et al., 1972). The concentration of tiobarbituric acid reactive substances (TBARSs) was measured with OxiTekTBARS kit (cat no. 850-287-KI01, Zeptometrix, Bufallo, NY, USA). The C-reactive proteins (CRP) were measured with a Rat CRP Elisa kit (cat no. RH 951CR-P01R Biovendor, Brno, Czech Republic). Activity of glutathione reductase (GSR) was measured in the serum of rats with a kit (cat no. GR 2368 Randox Laboratories Ltd., UK). In this method, GSR catalyses the reduction of glutathione in the presence of NADPH, which is oxidized to $NADP^+$. Activity of superoxide dismutase (SOD) was determined in erytocytes lysate with the use of a Randox kit (cat no. SD125 Randox Laboratories Ltd., UK). According to manual, xanthine and xanthine oxidase are used to generate superoxide radicals which react with 2-(4-iodophenyl-3-(4-nitrophenol)-5-phenyltetrazolium chloride to a form of a red formazan dye. Activity of HO-1 was measured accordingly to the Turcanu et al. (1998) method. $BaCl_2$ (0.250 g) was added to 0.500 ml of serum, vortexed for 15 sec., then 0.750 ml benzene was added. Samples were vortexed and centrifuged. The upper benzene layer was taken for measuring the absorbance at 450 nm. Results were shown as the concentration of bilirubin.

Crude lipids concentration in selected organs

The crude fat content was determined by the Soxhlet method with a Soxtec Avanti's 2050 Auto Extraction Unit (Tecator Foss, Hillerød, Sweden), (Fortuna et al., 2003). Samples were used for measurements of crude lipids content according to the application note of the Tecator Foss (ASN 3131) with slight modification concerning drying method (Kopeć et al., 2013). Petroleum ether was used to extract fat. Livers, kidneys and hearts were freeze-dried in lyophilizer (Christ Alpha 1-4, Gefriertrocknungsanlangen, Germany). After freeze-drying, organs were weighed, grounded and used for analysis.

Gene expression

mRNA was isolated with a commercially available kit (cat no. 610.12 Invitrogen Life Technologies, Norway) and its concentration was measured by a spectrophotometer at absorbance 260 and 280 nm. For cDNA synthesis mRNA was reverse transcribed with the use of a SuperScript® VILO cDNA Synthesis Kit (11754-050 Invitrogen Life Technologies, Norway). cDNA was subjected to real time PCR in a reaction of a mixture containing TaqMan Gene Expression Master mix and primers with fluorescent marked starters (Invitrogen, Life Technologies, Norway). The thermal profile of the PCR reaction included initial denaturation for 15 min at 95°C, followed by 40 amplification cycles of denaturation for 1 sec at 95°C, annealing for 20 sec at 60°C, and elongation for 20 sec at 72°C. RT-PCR reaction was performed with the use of the Applied Biosystems 7900HT Fast Real-Time PCR System. The expression rates were calculated as the normalized treshold cycle (C_T) difference between a control sample and a sample with the adjustment for the amplification efficiency relative to the expression level of the housekeeping gene *Sp1*.

Statistical analysis

The data was presented as mean ± SD. One-way, non-parametric analysis of variance (Statistica v. 8.1, StatSoft, Inc. 2007, Tulsa, OK, USA) was applied for testing the differences between experimental treatments. The Kruskal-Wallis test was used for the identification of statistically significant differences at a level of $p < 0.05$.

Results

Food intake, body weight gain and chosen organs weight

Food intake of rats fed experimental diets was not significantly different among experimental groups. Experimental diets in the presence or absence of RSV did not affect body weight gain of experimental rats. There were not differences in the liver weight of rats fed the negative control (NC) diet and other experimental diets. Kidneys and heart mass was also not affected by different dietary treatments (data not showed).

Table 2. Serum lipids, selected biochemical parameters concentration, activity of chosen antioxidant enzymes and their mRNA expression

Parameters	NC	PC	PC + RSV 0.05%	PC + RSV 0.1%
Lipid profile CRP and TBARS level				
TC, mmol · l^{-1}	2.16 ± 0.22^{a}	3.69 ± 0.44^{b}	2.11 ± 0.24^{a}	2.19 ± 0.49^{a}
HDL, mmol · l^{-1}	1.47 ± 0.18^{a}	1.34 ± 0.30^{ac}	1.24 ± 0.27^{ac}	1.16 ± 0.09^{b}
HDL/TC ratio	0.83 ± 0.13^{a}	0.44 ± 0.11^{b}	0.59 ± 0.15^{c}	0.55 ± 0.09^{c}
LDL, mmol · l^{-1}	0.31 ± 0.26^{b}	1.72 ± 0.44^{a}	0.86 ± 0.37^{c}	1.03 ± 0.49^{c}
TAG, mmol · l^{-1}	2.34 ± 0.52^{a}	2.36 ± 0.64^{a}	1.54 ± 0.59^{b}	2.15 ± 0.48^{a}
CRP, μg ml^{-1}	247.6 ± 18.8	250.6 ± 25.7	242.6 ± 16.4	248.2 ± 19.2
TBARS†, nmol· l^{-1}	36.06 ± 11.6^{a}	36.2 ± 10.5^{a}	28.7 ± 6.7^{bc}	18.9 ± 1.6bc
Selected antioxidant enzymes activity				
GSR, U · l^{-1}	47.3 ± 2.36^{a}	39.9 ± 7.05^{ac}	37.2 ± 12.0^{bc}	39.7 ± 1.99^{ac}
SOD,U · ml^{-1}**	122.5 ± 50.7	103.2 ± 51.7	91.9 ± 19.8	104.0 ± 48.3
HO-1, mmol · l^{-1} ††	1.96 ± 0.49	1.71 ± 0.32	1.60 ± 0.52	1.58 ± 0.38
Relative mRNA gene expression of selected antioxidant enzymes				
Gsr	1.84 ± 0.02	1.82 ± 0.02	1.86 ± 0.02	1.87 ± 0.01
Sod	1.32 ± 0.01	1.30 ± 0.06	1.28 ± 0.03	1.29 ± 0.01
Hmox1	1.87 ± 0.02	1.90 ± 0.04	1.90 ± 0.03	1.90 ± 0.03
Gpx	1.55 ± 0.01	1.49 ± 0.06	1.47 ± 0.03	1.48 ± 0.02

* SD – standard deviation; values in column with different letters (a, b, c) are significantly different, $p \leq 0.05$; **measured in lysate of erythrocytes; † expressed as the MDA equivalent; †† expressed as the amount of bilirubin mmol · l^{-1}; NC-AIN-93G diet; PC – hypercholesterolaemic diet; PC + 0.05% RSV – hypercholesterolaemic diet with addition of 0.05% RSV; PC + 0.1% RSV – hypercholesterolaemic diet with addition of 0.1% RSV; TC – total cholesterol, TAG – triacylglycerols, CRP – C-reactive proteins, TBARS – tiobarbituric acid reactive substances, GSR – glutathione reductase, SOD – superoxide dismutase, HO-1 – haeme oxygenase 1

Lipid profile, TBARs and CRP level

The level of TC was significantly decreased in the serum of rats fed with the addition of 0.05% and 0.1% of resveratrol compared to the group fed with the PC diet. There were no differences between the concentration of TC in the serum of rats fed with the NC diet and those fed with different levels of RSV (Table 2). HDL cholesterol was significantly lower in the serum of rats fed a diet with 0.1% of RSV compared to the NC group. LDL content was significantly lower in the serum of rats fed with the PC+RSV 0.05% and PC+RSV 0.1% diets compared to the PC group. TAG level was significantly lower in the serum of rats fed with 0.05% of RSV in comparison to other experimental groups. The concentration of CRP in the serum of rats fed with various experimental diets did not significantly differ.

The content of TBARSs (MDA equivalents) significantly decreased in the serum of animals fed diets with different level of RSV compared to the NC and PC groups.

Activity and mRNA expression of chosen antioxidant enzymes

SOD and HO-1 activity was not affected by a PC diet or the addition of RSV to that diet (Table 2). Activity of GSR was significantly lower in the serum of rats fed with the diet containing 0.05% of RSV compared to the NC group. mRNA expression of *Gsr*, *Sod*, *Hmox1* and *Gpx* was not affected by dietary treatment (Table 2).

Concentration of crude lipids in liver, heart and kidneys

After 8 weeks of the experiment the lowest content of crude lipids in the liver was determined in the group of animals fed with the diet containing 0.1% of RSV (14.97 ± 3.46 g ·100 g DM^{-1}) and the highest in livers of rats fed with the PC diet (18.88 ± 4.55 g · 100 g DM^{-1}) albeit these differences were not significant (Table 3). The concentration of total fat in the hearts of rats was the lowest in the group fed the diet with 0.1% of RSV, and the highest in the group of animals fed with NC diet (7.11 ± 0.64 g · 100 g DM^{-1}). Content of fat in hearts of rats fed with 0.05 or 0.1% of RSV was significantly lower compared to hearts of rats fed with NC diet. The significantly ($p < 0.05$) lowest total fat in the kidneys of animals was measured in the group fed with 0.05% and 0.1% of RSV compared to the group fed with the PC diet. There were no differences in the fat content in kidneys of animals fed with the RSV (both levels) and the NC diet.

Table 3. Concentration of crude lipids in selected organs of rats, %

Treatment	Heart	Liver	Kidney
NC	8.44 ± 3.50^{b}	13.5 ± 3.90	13.6 ± 1.66^{a}
PC	5.94 ± 0.24^{a}	18.9 ± 4.55	15.8 ± 1.64^{b}
PC + RSV 0.05%	5.55 ± 0.55^{a}	18.4 ± 6.07	13.9 ± 1.97^{a}
PC + RSV 0.1%	5.34 ± 0.53^{a}	14.9 ± 3.46	13.3 ± 2.18^{a}

* SD – standard deviation; values in column with different letters (a, b, c) are significantly different, $p \leq 0.05$; NC – AIN-93G diet; PC – hypercholesterolaemic diet; PC+0.05% RSV – hypercholesterolaemic diet with addition of 0.05% RSV; PC+0.1% RSV – hypercholesterolaemic diet with addition of 0.1% RSV

Discussion

In this study the effects of RSV on food intake, body weight gain and organs weight of rats (data not showed) were not found. These results indicate that in terms of growth, the doses of resveratrol used in this study were tolerated by the rats. Our results correspond with data published by other authors. Aubin et al. (2008) reported also, that food intake, body weight gain, liver and heart weight of rats was not affected by resveratrol (20 $mg \cdot kg^{-1} \cdot day^{-1}$) added to a high fat diet (42% lipids, 36% carbohydrates and 22% protein). Juan et al. (2002) showed that the 28-day oral administration of RSV in high dose (20 $mg \cdot kg^{-1}$ body weight), has no effect on the body weight gain of rats fed with a rodent commercial diet, containing 16.04% protein, 2.6% fibre, 46.74% carbohydrate, 2.95% lipids and 4.22% ash. They also did not report any effect of RSV on food intake. Tauriainen et al. (2011) found that both low and high content of RSV (2 $mg \cdot kg^{-1}$ or 4 $mg \cdot kg^{-1}$ diet, respectively) in the diet have no effect on body weight gains of mice. Albeit in some animal studies, especially with mice different models, it was shown that RSV reduces body weight gain or may affect the weight of organs (Ahn et al., 2008; Aribal et al., 2009; Kim et al., 2011; Kitada et al., 2011). Ahn et al. (2008) reported that mice fed atherogenic diet (1% cholesterol, 8% cocoa butter and oil) with 0.0125% RSV had lower body gain and liver weight compare to control animals. These authors did not reported intake of diets during the experiment. Kim et al. (2011) reported that male C57BL/6 mice fed with a diet containing 20% of fat (17% lard and 3% maize oil), 1% of cholesterol and 0.4% RSV had lower body gain without affecting food intake. It can be suggested that the lowering body gain effect of RSV depend on used dose of it and animal model.

The level of TC was significantly decreased ($p < 0.05$) in the serum of rats fed with different levels of RSV compared to the serum of animals fed with the PC diet (Table 2). It has been previously reported that RSV may inhibit certain enzymes in cholesterol synthesis and mRNA gene expression involved in the lipid and sterol metabolism (Ahn et al., 2008; Kim et al., 2011; Azorín-Ortuño et al., 2012). It can be suggested that, in our study, RSV added to the PC diet could inhibit cholesterol synthesis. It could result in deficiencies of endogenous cholesterol and the one from the diet that was used for the synthesis of bile acids, hormones and the other compounds. Ahn et al. (2008) reported that the level of TC in the plasma was significantly increase in mice fed atherogenic diet (1% cholesterol, 8% cocoa butter and oil) compared to the animals fed control diet (AIN-76). These authors have shown that addition to the atherogenic diet of the RSV (0.0125%) significantly decreased content of TC in plasma. They reported that the expression of genes involved in cholesterol synthesis (*Sqle*, *Fdft1*) was lower in the liver of mice fed the high-fat diet supplemented with RSV and it resulted in a lower cholesterol synthesis and reduced level in the serum. Additionally, Penumathsa et al. (2008) reported that plasma cholesterol significantly decreased in rats fed with the RSV (20 $mg \cdot kg^{-1}$) added to hypercholesterolaemic diet (5% cholesterol). Results obtained in this study are similar to data published by other authors (Kim et al., 2011, Schmatz et al., 2011). In contrast, Juan et al. (2002) did not find differences in the serum cholesterol between the control group and the one that was enriched with a high dose of RSV. Furthermore, the results of experiments conducted by Aubin et al. (2008) showed no significant differences in total cholesterol levels between experimental groups.

The concentration of HDL-cholesterol was significantly lower in the serum of rats fed with 0.1% RSV compared to other experimental groups, additionally the level of TC and LDL-cholesterol was also decreased. Probably it caused the lower level of HDL (Table 2). Rats naturally have high level of HDL and addition to the diet cholesterol and its sources (e.g. butter, lard) decreases the level of HDL and elevates total and LDL-cholesterol (Pisulewski et al., 2002). It was confirmed in this study. RSV lowering TC levels, also affected the reduction of the HDL cholesterol, but the ratio of HDL/TC cholesterol significantly increased in groups of rats fed with 0.05 or 0.1% RSV compared to PC group, which was important finding of this study. Ahn et al. (2008) and Aubin et al. (2008) did not find any effect of RSV on HDL level in the plasma of rats.

LDL cholesterol concentration was significantly ($p<0.05$) lower in the serum of rats fed with 0.05% and 0.1% of RSV compared to the serum of rats fed with the PC diet (Table 2). Hypercholesterolaemic diet significantly increases cholesterol level in blood, resulting in the risk of cardiovascular diseases. Supplementation of the diet with RSV may reverse these effects. We also found that the most effective dose in reduction of LDL was at the level of 0.05% of RSV in diet. Additionally, this dose significantly ($p<0.05\%$) decreased the TAG level in serum of rats compared to other experimental groups (Table 2). Kim et al. (2011) showed that TAG content was lower in the plasma of mice fed with the high-fat diet in the presence of RSV (0.4%).

Schmatz et al. (2011) also demonstrated that the addition of RSV to drinking water (10 mg · kg^{-1} and 20 mg · kg^{-1}) resulted in a significant reduction in TAG levels in diabetic rats. In contrast, Juan et al. (2002) and Aubin et al. (2008) showed that oral administration of RSV did not affect the LDL, and TAG concentration in the plasma of experimental rats.

The CRP concentration in the serum of rats was not affected by a high-fat diet or addition of RSV to this diet. Kaur et al. (2007) showed that RSV in dose-depended manner suppressed the cytokine-induced CRP expression in Hep3B cells. CRP is the acute phase plasma protein that can be used as a marker not only for inflammation but can also be used as a proatherogenic factor. It was reported that CRP promotes atherotrombosis and coagulation. The level of this protein also increases in progression in some chronic diseases (diabetes, cardiovascular events, inflammatory bowel diseases, infections) (Kaur et al., 2007; Coventry et al., 2009).

In this study it was also found that TBARSs were significantly decreased in serum of rats fed PC diet with both doses of RSV (Table 2). It has been reported previously that diets containing various level of cholesterol (0.05%, 0.5%, 1%) and various type of fat (butter, beef tallow, soya bean oil) increases oxidative stress in rat model (Yuan et al., 1998; Yuan and Kitts, 2003; Rocha et al., 2009). It was also reported that RSV decreases concentration of various oxidative products of lipid peroxidation in rats fed high cholesterol high fat diet (Rocha et al., 2009). Probably this effect is connected with strong antioxidant activity of RSV. We did not found similar data concerning the effect of resveratrol on the TBARS concentration in the serum of rats fed diet with cholesterol in available literature.

Activities of SOD and HO-1 tended to be lower in rats fed with RSV, but in case of SOD only the dose of 0.05% of the RSV given this effect (Table 2). Activity of GSR was significantly ($p < 0.05$) lower in the serum of rats fed the PC diet with 0.05% of RSV compared to the NC group. We also did not find any effect in different doses of RSV on mRNA *Gpx*, *Sod*, *Gsr* and *Ho-1* expression in the liver. Diet with the addition of cholesterol and butter increases oxidative stress in the organism and probably RSV as the high activity antioxidant scavenged lipid free radicals, and activities of antioxidant enzymes were not affected. These results do not correspond with data reported by other authors and this is probably connected with a lower dose of RSV used in their studies. Rocha et al. (2009) showed higher activity of SOD, catalase or superoxide dismutase in liver of rats fed a high fat diet in presence of RSV (1 mg · kg^{-1} body weight per day). It was also reported that RSV in human hepatoma cells (HUH7) and in rats induces the HO-1 enzyme (Wagner et al., 2011). This was not confirmed in our study.

Total fat content in the hearts and the livers of experimental rats was not affected by different dietary treatment, nevertheless it tended to be lower in groups fed with RSV ($p = 0.06$, $p = 0.07$, respectively). Significantly lower total fat content was measured in kidneys of rats fed with 0.05% or 0.1% RSV compared to the PC group and did not differ from the NC group (Table 3). These results confirmed that RSV modulates lipid metabolism not only in livers and blood, but also in other organs. Accumulation of lipids in organs and tissues increases oxidative stress which results in the production of pro-inflammatory cytokines and chronic inflammation. For many years inflammation has been associated with microorganism's infections, but now it is well known, that it is also involved in chronic diseases (Leiro et al., 2010; Kim et al., 2011). Presence of RSV in diet may decrease accumulation of lipids and protects from non-communicable diseases. In literature usually the concentration of TC and TAG was determined in the livers or adipose tissue in rodents treated with different diets and level of RSV (Ahn et al., 2008; Macarulla et al., 2009). Lagouge et al. (2006) showed that the 0.4% addition of RSV to a high fat diet contributed to the reduction of adipose tissue. Similar results were reported by Kim et al. (2011) and Cho et al. (2012). Albeit not all studies confirm that RSV reduces body fat (Louis et al., 2011; Tauriainen et al., 2011).

Conclusions

Results obtained in this study showed that resveratrol (RSV) has strong hypocholesterolaemic effects and may decrease lipid concentration in different organs. Activities of antioxidant enzymes and their mRNA gene expression were not affected by resveratrol RSV. Using relatively high doses of RSV (0.05% and 0.1%) was sufficient to lowering oxidative stress without involving naturally occurring mechanisms in rats (glutathione reductase, glutathione peroxidase, superoxide dismutase, haeme oxygenase 1).

Ackowlegements

Authors are grateful to Antoni Borgiasz for his help during the animal studies.

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