

# Hypolipidemic and antioxidant effects of hydroxycinnamic acids, quercetin, and cyanidin 3-glucoside in hypercholesterolemic erythrocytes (in vitro study)

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## Abstract

**Objective** Four polyphenols: ferulic acid and *p*-coumaric acid (hydroxycinnamic acids), quercetin (flavonol) and cyanidin 3-glucoside (anthocyanin) were selected, and their antioxidant properties and their influence on cholesterol concentration in hypercholesterolemic and normal erythrocytes were investigated.

**Methods** To determine the effect of phenolic compounds, we prospectively studied cholesterol concentration, lipid peroxidation and membranes fluidity. Whole-blood and isolated erythrocytes (2% hematocrit) were incubated for 24 h with selected compounds at concentration 1, 10 and 100  $\mu\text{mol/L}$ . All investigated compounds decreased lipid peroxidation in whole blood. Cyanidin 3-glucoside and quercetin showed higher antioxidant properties than hydroxycinnamic acids (ferulic acid and *p*-coumaric acid).

**Results** Incubation of whole blood of hypercholesterolemic patients with quercetin and cyanidin 3-glucoside resulted in statistically significant reduction of cholesterol concentration in erythrocytes down to 75% (at 10  $\mu\text{mol/L}$  of polyphenols) and 69% (at 100  $\mu\text{mol/L}$  of polyphenols) of initial values. The effect of both compounds on isolated

erythrocytes was even more pronounced, reduction down to 70% (at 10  $\mu\text{mol/L}$  of polyphenols) and 58% (at 100  $\mu\text{mol/L}$  of polyphenols) of initial values. After incubation of isolated erythrocytes of hypercholesterolemic patients with quercetin and cyanidin 3-glucoside, increase of membrane fluidity was noticed. After incubation of isolated erythrocytes of healthy donors with investigated compounds, no changes in membrane fluidity were observed.

**Conclusion** Our results indicate that flavonols and anthocyanins have higher antioxidant properties and higher influence on cholesterol concentration in erythrocytes membranes than simple hydroxycinnamic acids.

**Keywords** Hypercholesterolemia · Lipid peroxidation · Cholesterol · Quercetin · Cyanidin 3-glucoside · Ferulic acid · Coumaric acid · Membrane fluidity

## Introduction

Phenolic compounds are a large group of secondary metabolites widespread in plant kingdom, and therefore, they are an integral part of our diet [1–3]. They are categorized into several classes depending on their structure and subcategorized within each class according to the degree of hydroxylation and the presence of other substituents. Phenolic compounds are divided into four major groups: lignans (C6–C4–C6), phenolic acids [hydroxybenzoic acids (C6–C1) and hydroxycinnamic acids (C6–C3)], flavonoids (C6–C3–C6) and stilbenes (C6–C2–C6) [4].

Hydroxycinnamic acids occur in foods and beverages as esters, amides and glycosides [5]. Most of flavonoids have been found in plants usually as *o*- $\beta$ -glycosides [6, 7].

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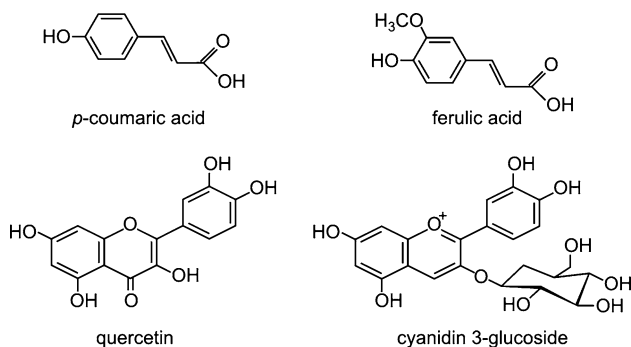
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In addition, anthocyanins may also exist in the acylated form with an ester linkage between a sugar and aliphatic or aromatic acid molecule [8].

Many phenolic compounds have been found to be strong free radicals scavengers and antioxidants. Antioxidant activity of flavonols, flavanols, anthocyanins and phenolic acids far exceeds that of well-known antioxidant vitamins (C and E) and carotenoids [9]. A number of highly reactive oxygen species are regularly produced in our body. An excessive free radical production, decrease in the activity of endogenous antioxidant enzymes and/or the low content of exogenous antioxidants can lead to oxidative stress [10]. The oxidation processes induced by free radicals can result in damages of lipids, proteins and nucleic acids, which are linked to the etiology and pathophysiology of many chronic diseases including cardiovascular disease [11].

Phenolic compounds tested in our study belong to hydroxycinnamic acids (ferulic and *p*-coumaric acids), flavonols (quercetin) and anthocyanins (cyanidin 3-glucoside) (Fig. 1). The ferulic and *p*-coumaric acids are rarely present as the free acid but occur in the plants predominantly in esterified form with quinic acid, glucose or tartaric acid [3]. Generally, ferulic acid is frequently less abundant than *p*-coumaric acid. Free hydroxycinnamic acids may appear in fruits and vegetables during the technological processing or in situation when plants display physiological disturbances or when they are contaminated by microorganisms [12]. Ferulic and *p*-coumaric acid derivatives were identified in citrus, black currant, strawberry, black mulberries, sweet and sour cherry [13–16]. In addition, feruoyl and coumaroyl esters of anthocyanins were found in red cabbage, and red radish [17, 18]. Cyanidin 3-glucoside is an anthocyanin most commonly found in fruits. It is the major pigment in blackberry, peach, elderberry and mulberry [12]. Quercetin glycosides are present nearly in all fruits and vegetables. Onions, kale, broccoli, beans, apple, black currants, apricot and cranberry are especially rich sources of quercetin glycosides [6, 19].



**Fig. 1** Structure of selected phenolic compounds

Epidemiological studies clearly indicate that diets with a high intake of whole grain cereals, vegetables and fruits may reduce the incidence of cardiovascular disease [20–22]. Plant-derived foods and beverages are rich in phenolic compounds which show protection properties against cancer, cardiovascular disease and aging [23].

Changes in human behavior and lifestyle over the last century have resulted in a dramatic increase in the occurrence of cardiovascular disease and hypertension. These illnesses are correlated with higher concentration of cholesterol in serum, especially with cholesterol in low-density lipoprotein (LDL) fraction [24]. One of the reasons of abnormal concentration of cholesterol is a lipid-rich diet. Another reason is our genetic proneness to accumulation of store substances or our damaged genetic material.

Cholesterol is one of the essential structural and functional components of cell membrane. It has been reported that the concentration of cholesterol in the erythrocyte membrane is increased in hyperlipidemia patients [25–27]. Higher concentration of cholesterol in erythrocyte membrane causes a decrease in the deformability of red blood cells, which may lead to impairment of the hemorheological behavior that can promote atherosclerosis [25, 28]. One of the mechanisms responsible for the changes in erythrocytes in hypercholesterolemia has been reported to be oxidative stress [29].

The aim of the present study was to investigate the effect of four phenolic compounds (ferulic acid, *p*-coumaric acid, quercetin and cyanidin 3-glucoside) on cholesterol concentration, lipid peroxidation and membranes fluidity in hypercholesterolemic and normal erythrocytes.

## Materials and methods

### Chemicals

Ferulic acid, *p*-coumaric acid, quercetin and 5-doxydstearic acid (5-DSA) were obtained from Sigma–Aldrich (USA) and cyanidin 3-glucoside from Extrasynthese (France). All other chemicals and reagents were obtained from POCh (Gliwice, Poland) and were of analytical grade.

### Materials and incubation conditions

Blood from hypercholesterolemic patients (total cholesterol concentration (TC) > 250 mg/dL, LDL cholesterol (LDL-C) > 160 mg/dL, triglycerides (TG) < 400 mg/dL) and from healthy donors (TC < 200 mg/dL, LDL-C < 135 mg/dL, TG < 200 mg/dL) were obtained from the Department of Clinical Pharmacology, Medical University of Lodz. Blood was collected with anticoagulant (23 mM citric acid, 45.1 mM trisodium citrate, 45 mM

glucose) in 5:1 ratio. The study involved 40 patients with type-2 hypercholesterolemia (17 men and 23 women, mean age  $55.9 \pm 7.4$ ). The control group consisted of 20 healthy individuals (9 men and 11 women, mean age  $50.3 \pm 8.2$ ). These experiments were carried out in accordance with the ethics standards as formulated in The Helsinki Declaration of 1975 (revised 1983); consent number 385/05/KB of Commission of Medical Research Ethics of Medical University of Łódź, Poland.

Erythrocytes were washed three times in phosphate-buffered 0.9% NaCl (pH 7.4) and centrifuged at  $600 \times g$ . Erythrocytes were suspended in incubation medium (140 mM NaCl, 10 mM KCl, 1.5 mM MgCl<sub>2</sub>, 10 mM glucose, 10 mM HEPES, 100 µg/mL streptomycin, 5 mM TRIS–HCl buffer, pH 7.4) at a hematocrit of 2%.

Whole blood was incubated for 24 h at 37 °C without and with phenolic compounds at concentration 1, 10 and 100 µmol/L. Concentration of investigated compounds in isolated erythrocytes was recalculated according to hematocrit of blood and final concentrations were 0.045, 0.45 and 4.5 µmol/L. All compounds were dissolved in incubation medium. After incubation, erythrocytes were washed three times in buffered 0.9% NaCl.

#### Red cell membrane preparation

The erythrocyte membranes were prepared by the method of Dodge et al. [30] with Tris–HCl buffer. The protein concentration was estimated according to the Lowry methods [31].

#### Extraction of lipids

The lipid extraction from erythrocyte membranes was carried out with organic solvent of small toxicity according to the method of Rodriguez-Vico et al. [32]. Erythrocytes were placed in a flat-bottomed flask, and they were shaken with a hexane and 2-propanol mixture (3:2). Everything was centrifuged at  $4,000 \times g$  for 15 min. The supernatant was transferred into dry flask. The flask was connected to the vacuum evaporator in order to evaporate solvents. Dry lipids were solved in a mixture of ethanol/chloroform (9:1, v/v).

#### Cholesterol concentration

The concentration of cholesterol was marked on the basis of the intensity of green color, which was made in the vicinity of glacial acetic acid, acetic anhydride and concentrated sulfuric acid—the reaction of Liebermann-Burchard [33]. Acetic anhydride and concentrated sulfuric acid dehydrate the cholesterol molecule in anhydrous conditions. It results in setting an additional double bond,

double bond-linked present in cholesterol molecule. Concentration of cholesterol was expressed as milligrams of cholesterol/mL packed cells (mg HC/mL packed cell).

#### Peroxidation of lipids (TBARS)

The peroxidation of erythrocyte lipids was measured by the thiobarbituric acid method [34, 35]. Absorbance of color reaction products was measured with a spectrophotometer at 532 nm. The concentration of hemoglobin was determined using the method by Drabkin [36]; absorbance was measured with a spectrophotometer (540 nm). Lipid peroxidation was expressed as micromoles of thiobarbituric acid-reactive substances (TBARS) per gram hemoglobin (Hb) (µmol TBARS/g Hb).

#### Conjugated dienes

Concentration of conjugated dienes was determined in the lipid extracts from erythrocyte membranes. Chloroform/methanol mixture was removed under non-oxygen condition, and lipids were dissolved in cyclohexane. The spectrophotometric absorption was measured at 233 nm after 5-min incubation according to Buege and Aust [37]. Concentration of conjugated dienes was expressed as absorbance units (AU)/milligram proteins.

#### Membrane fluidity

Fluidity of erythrocyte membranes was determined by means of electron paramagnetic resonance (EPR) spectroscopy with using 5-DSA spin label. EPR measurements were performed in a Bruker 300 Spectrometer (Germany). Order parameter *S* was calculated as described in Koter et al. [25].

#### Statistical analysis

Significance of differences between healthy and disease groups was calculated by using unpaired Student *t* test. Multiple comparisons were made by ANOVA and Tukey's a posteriori test (calculations made with STATISTICA<sup>®</sup> 6.1, StatSoft Inc., Tulsa, USA).

## Results

Table 1 contains the initial values of cholesterol concentration, level of lipid peroxidation, concentration of conjugated dienes and order parameter *S* in control and disease group. As expected, the erythrocytes from hyperlipidemic patients showed higher values for each parameter.

**Table 1** The initial parameters of erythrocytes

Parameter	Healthy donors	Hyperlipidemia patients
Cholesterol concentration (mmol/mL packed cells)	2.09 ± 0.18 ( <i>n</i> = 20)	3.87 ± 0.23* ( <i>n</i> = 30)
Lipid peroxidation (μmol TBARS/mg Hb)	0.157 ± 0.038 ( <i>n</i> = 20)	0.280 ± 0.040* ( <i>n</i> = 30)
Concentration of conjugated dienes (AU/g proteins)	0.073 ± 0.005 ( <i>n</i> = 10)	0.209 ± 0.018* ( <i>n</i> = 20)
Order parameter S	0.742 ± 0.004 ( <i>n</i> = 10)	0.756 ± 0.006* ( <i>n</i> = 30)

\* *t* Student unpaired test,  
*P* < 0.001

**Table 2** Concentration of cholesterol (μmol CH/mL packed cells) in erythrocytes after 24 h incubation of whole blood

Concentration of polyphenols (μmol/L)	Healthy donors ( <i>n</i> = 20)	Disease donors ( <i>n</i> = 30)
Before incubation	2.09 ± 0.18	3.70 ± 0.23
After incubation	1.99 ± 0.21	3.68 ± 0.22
Ferulic acid		
1	2.10 ± 0.16	3.67 ± 0.22
10	1.96 ± 0.14	3.61 ± 0.20
100	1.91 ± 0.16	3.54 ± 0.26
<i>p</i> -Coumaric acid		
1	2.00 ± 0.16	3.65 ± 0.21
10	1.97 ± 0.17	3.62 ± 0.19
100	1.93 ± 0.18	3.57 ± 0.24
Quercetin		
1	1.98 ± 0.15	3.45 ± 0.31
10	1.92 ± 0.15	2.78 ± 0.27 <sup>a,b</sup>
100	1.85 ± 0.17	2.54 ± 0.21 <sup>a,c</sup>
Cyanidin 3-glucoside		
1	1.97 ± 0.14	3.48 ± 0.25
10	1.93 ± 0.15	2.75 ± 0.30 <sup>a,b</sup>
100	1.87 ± 0.13	2.58 ± 0.23 <sup>a,c</sup>
ANOVA	Statistically no significant	<i>P</i> < 0.001

<sup>a</sup> *P* < 0.001 versus before incubation

<sup>b</sup> *P* < 0.05 versus concentration of 1 μmol/L

<sup>c</sup> *P* < 0.05 versus concentration of 10 μmol/L

**Table 3** Concentration of cholesterol (μmol CH/mL packed cells) in erythrocytes after 24 h incubation of isolated erythrocytes

Concentration of polyphenols (μmol/L)	Healthy donors ( <i>n</i> = 20)	Disease donors ( <i>n</i> = 30)
Before incubation	2.16 ± 0.18	3.87 ± 0.21
After incubation	1.99 ± 0.21	3.84 ± 0.18
Ferulic acid		
1	2.13 ± 0.17	3.79 ± 0.21
10	2.06 ± 0.18	3.62 ± 0.16
100	1.96 ± 0.21	3.51 ± 0.18
<i>p</i> -Coumaric acid		
1	2.15 ± 0.14	3.78 ± 0.19
10	2.04 ± 0.18	3.64 ± 0.17
100	1.97 ± 0.16	3.49 ± 0.21
Quercetin		
1	2.09 ± 0.11	3.69 ± 0.19
10	1.91 ± 0.21	2.61 ± 0.25 <sup>b,c</sup>
100	1.82 ± 0.19 <sup>a</sup>	2.21 ± 0.16 <sup>b,d</sup>
Cyanidin 3-glucoside		
1	2.10 ± 0.12	3.70 ± 0.22
10	1.89 ± 0.16	2.70 ± 0.19 <sup>b,c</sup>
100	1.81 ± 0.20 <sup>a</sup>	2.23 ± 0.21 <sup>b,d</sup>
ANOVA	<i>P</i> < 0.05	<i>P</i> < 0.001

<sup>a</sup> *P* < 0.05 versus before incubation

<sup>b</sup> *P* < 0.001 versus before incubation

<sup>c</sup> *P* < 0.001 versus concentration of 1 μmol/L

<sup>d</sup> *P* < 0.05 versus concentration of 10 μmol/L

Incubation of whole blood of hypercholesterolemic patients with quercetin and cyanidin 3-glucoside resulted in statistically significant reduction of cholesterol concentration in erythrocytes down to 75% (at 10 μmol/L of polyphenols) and 69% (at 100 μmol/L of polyphenols) of initial values (Table 2). The effect of both compounds on isolated erythrocytes was even more pronounced, reduction down to 70% (at 10 μmol/L of polyphenols) and 58% (at 100 μmol/L of polyphenols) of initial values (Table 3).

Incubation with ferulic acid and *p*-coumaric acid was not effective for whole blood (Table 2) while 10% cholesterol level reduction was observed for isolated red blood cells for both acids (at concentration of 100 μmol/L) (Table 3).

For healthy donors, a slight reduction of cholesterol levels was observed. Quercetin and cyanidin 3-glucoside,

at concentration of 100 μmol/L, significantly reduced cholesterol by 10% in erythrocytes after incubation of isolated erythrocytes (Tables 2, 3).

After incubation without polyphenols, an increase in lipid peroxidation was observed for healthy donors and hypercholesterolemic patients, for whole blood by 30% and for isolated erythrocytes by 50%. All investigated compounds showed antioxidant effects in both experimental systems.

After incubation of whole blood of hypercholesterolemic patients with quercetin and cyanidin 3-glucoside, significant reduction of lipid peroxidation in erythrocytes was observed (Table 4). The similar effect of both compounds was noticed after incubation with isolated erythrocytes (Table 5). Ferulic acid and *p*-coumaric acid significantly reduced lipid peroxidation at concentrations 10 and 100 μmol/L in both experimental systems (Tables 4, 5).

**Table 4** Thiobarbituric acid-reactive substances level ( $\mu\text{mol}$  TBARS/mg Hb) in erythrocytes after 24 h incubation of whole blood

Concentration of polyphenols ( $\mu\text{mol/L}$ )	Healthy donors ( $n = 20$ )	Disease donors ( $n = 30$ )
Before incubation	$0.157 \pm 0.028$	$0.281 \pm 0.040$
After incubation	$0.207 \pm 0.021$	$0.365 \pm 0.023$
Ferulic acid		
1	$0.199 \pm 0.018$	$0.312 \pm 0.021^a$
10	$0.191 \pm 0.019^a$	$0.300 \pm 0.016^b$
100	$0.185 \pm 0.021^a$	$0.287 \pm 0.018^b$
p-Coumaric acid		
1	$0.200 \pm 0.021$	$0.311 \pm 0.019^a$
10	$0.195 \pm 0.018^a$	$0.300 \pm 0.017^b$
100	$0.186 \pm 0.015^a$	$0.291 \pm 0.021^b$
Quercetin		
1	$0.193 \pm 0.011^a$	$0.299 \pm 0.019^a$
10	$0.161 \pm 0.018^b$	$0.290 \pm 0.025^b$
100	$0.158 \pm 0.019^b$	$0.281 \pm 0.016^b$
Cyanidin 3-glucoside		
1	$0.194 \pm 0.012^a$	$0.298 \pm 0.022^a$
10	$0.163 \pm 0.016^b$	$0.286 \pm 0.019^b$
100	$0.157 \pm 0.020^b$	$0.279 \pm 0.021^b$
ANOVA	$P < 0.001$	$P < 0.001$

<sup>a</sup>  $P < 0.05$  versus after incubation

<sup>b</sup>  $P < 0.001$  versus after incubation

Concentration of conjugated dienes was measured before and after incubation of isolated erythrocytes with investigation compounds. After incubation without polyphenols, an increase in concentration of conjugated dienes was observed for healthy donors and hypercholesterolemic patients, for whole blood by 37% and for isolated erythrocytes by 44%. All investigated compounds showed protecting effects in both experimental systems (Fig. 2). Higher changes were observed for quercetin and cyanidin 3-glucoside.

Membrane fluidity at the depth of the fifth carbon atom in the fatty acid chains of phospholipids was determined by using 5-DSA spin label in isolated erythrocytes. After incubation of the healthy group with polyphenols, no changes in order parameter S were observed. Quercetin and cyanidin 3-glucoside, at concentrations 10 and 100  $\mu\text{mol/L}$ , significantly reduced order parameter S (increase membrane fluidity) (Fig. 3).

## Discussion

Detailed mechanisms by which phenolic compounds prevent from coronary heart diseases are uncertain. Phenolic compounds may reduce the formation of free radicals, may inhibit oxidation of low-density lipoproteins (LDL) by

**Table 5** Thiobarbituric acid-reactive substances level ( $\mu\text{mol}$  TBARS/mg Hb) in erythrocytes after 24 h incubation of isolated erythrocytes

Concentration of polyphenols ( $\mu\text{mol/L}$ )	Healthy donors ( $n = 20$ )	Disease donors ( $n = 30$ )
Before incubation	$0.150 \pm 0.017$	$0.251 \pm 0.037$
After incubation	$0.225 \pm 0.012$	$0.361 \pm 0.036$
Ferulic acid		
1	$0.224 \pm 0.018$	$0.351 \pm 0.016$
10	$0.199 \pm 0.011^a$	$0.309 \pm 0.032^a$
100	$0.179 \pm 0.016^b$	$0.279 \pm 0.021^b$
p-Coumaric acid		
1	$0.223 \pm 0.020$	$0.349 \pm 0.019$
10	$0.210 \pm 0.019^a$	$0.311 \pm 0.032^a$
100	$0.181 \pm 0.017^b$	$0.283 \pm 0.021^b$
Quercetin		
1	$0.205 \pm 0.018^a$	$0.335 \pm 0.019^a$
10	$0.181 \pm 0.019^b$	$0.299 \pm 0.023^b$
100	$0.151 \pm 0.022^b$	$0.250 \pm 0.019^b$
Cyanidin 3-glucoside		
1	$0.209 \pm 0.018^a$	$0.341 \pm 0.018^a$
10	$0.182 \pm 0.011^b$	$0.300 \pm 0.016^b$
100	$0.150 \pm 0.018^b$	$0.251 \pm 0.018^b$
ANOVA	$P < 0.001$	$P < 0.001$

<sup>a</sup>  $P < 0.05$  versus after incubation

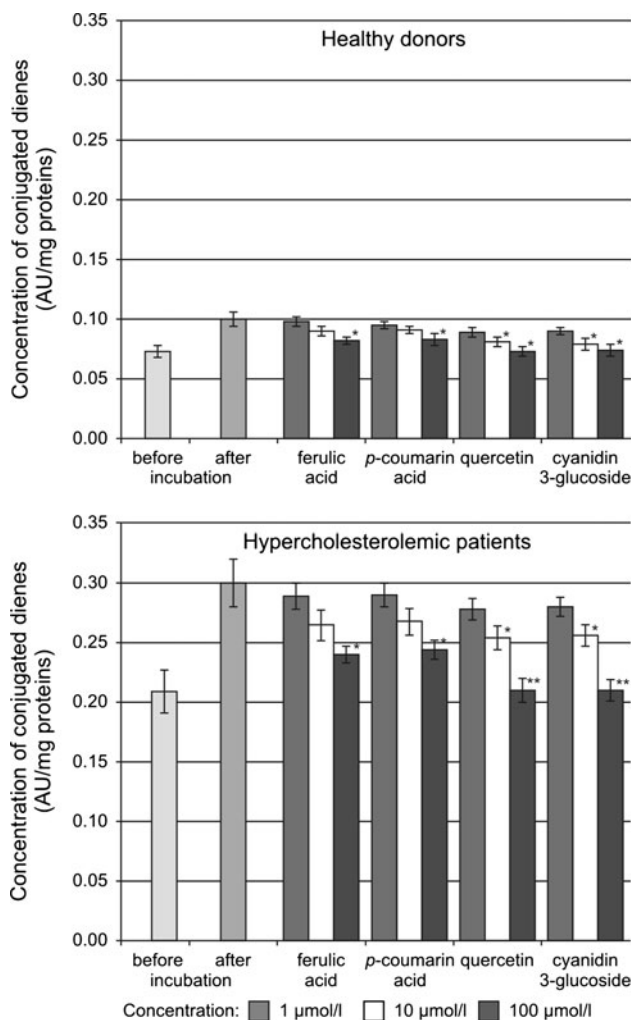
<sup>b</sup>  $P < 0.001$  versus after incubation

chelating divalent metal ions and may also inhibit platelet aggregation and adhesion [38–42]. In addition, flavonoids showed a protective activity to  $\alpha$ -tocopherol in human LDL, and they can also regenerate vitamin E, from the  $\alpha$ -chromanoxyl radical [43–45].

Several studies have shown that phenolic compounds reduced in vitro oxidation of LDL, a factor contributing to the development of atherosclerosis [9, 46–49].

In our study, we observed higher lipid peroxidation in hypercholesterolemia erythrocytes (Table 1). This is a consequence of increased production of free radicals. Mosinger [50] found that higher concentration of cholesterol in LDL causes an acceleration of its oxidation.

Many authors have demonstrated that under in vitro conditions, effectiveness of the phenolic compounds in protecting LDL from oxidation depends on their stability as well as on number and location of hydroxyl groups [51, 52]. Generally, phenolic acids and flavonoids with ortho-dihydroxy structure are the most efficient antioxidants. Probably, for this reason epigallocatechin gallate, which has four ortho-dihydroxy groups, has been found as the most potent antioxidant among 32 phenolic compounds tested by Vinson et al. [9]. Also other studies with hydroxycinnamic acids confirm above-mentioned dependence [53, 54]. Hsieh et al. [55] demonstrated that phenolic



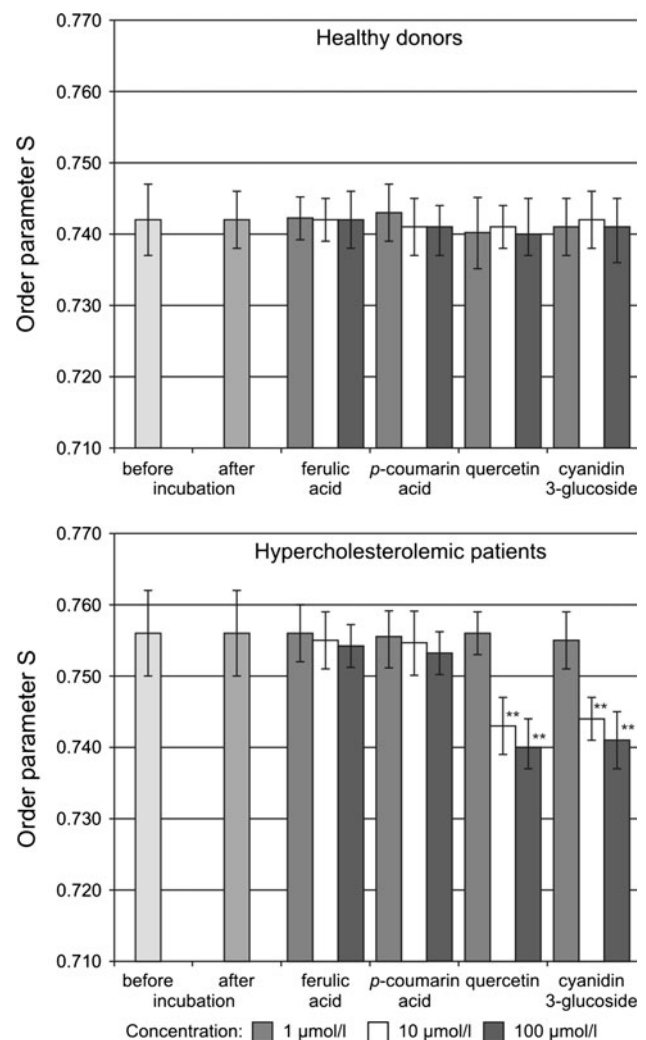
**Fig. 2** Changes in concentration of conjugated dienes after 24 h incubation of isolated erythrocytes with polyphenols. \* $P < 0.05$  versus after incubation, \*\* $P < 0.001$  versus after incubation

acids possess also a strong antioxidant activity, which decreases in LDL and erythrocyte membranes effects of UVB-induced peroxidation.

Our results showed higher protective properties for anthocyanidins and flavonols than for hydroxycinnamic acids, being associated with their chemical structure (Tables 4, 5, Fig. 2).

In addition, a protective role of phenolic compounds against oxidative damage of blood plasma proteins has been suggested in various in vivo studies. These protective effects have been observed for proanthocyanidins from grape seeds [56, 57], catechin, quercetin and resveratrol present in red wine [58] and catechins from tea [59]. Moreover, epidemiological studies have provided evidence that a high consumption of phenolic compounds is inversely related to the risk of cardiovascular diseases [60–62].

In vivo studies on rats and rabbits indicated that hydroxycinnamic acids and their derivatives lowered the



**Fig. 3** Changes in order parameter S after 24 h incubation of isolated erythrocytes with polyphenols. \*\* $P < 0.001$  versus after incubation

plasma cholesterol and could inhibit atherosclerogenesis [63–65]. Baicalin is a flavone which has shown antioxidant properties and also decreased cholesterol concentration in erythrocyte membranes in vitro [66]. Arai et al. [67] proved inverse correlation between quercetin intake and plasma total cholesterol concentration and plasma LDL-cholesterol concentration.

In our study, we observed a decrease in cholesterol concentration in erythrocyte membranes after 24 h incubation of whole blood or isolated erythrocytes with investigated compounds. Higher changes after isolated erythrocyte incubation in comparison with whole-blood incubation may be caused by cholesterol absence in incubation medium. Erythrocytes in whole blood can rebuild cholesterol from plasma during the 24 h incubation.

Larmo et al. [68] showed that flavonols from sea buckthorn berries did not affect the serum total, HDL and LDL cholesterol concentration in healthy adults. The

present study demonstrates that quercetin and cyanidin 3-glucose are effective in reducing cholesterol concentration in erythrocytes of hypercholesterolemic patients, while no change was observed in the control group.

Although, cholesterol is an essential structural and functional component of cell membranes, higher levels of cholesterol decrease the fluidity of erythrocyte membranes. Order parameter *S* is inversely proportional to membrane fluidity. After incubation of isolated erythrocytes of hypercholesterolemic patients with quercetin and cyanidin 3-glucose, increase of membrane fluidity (decrease in order parameter *S*) was observed. No changes in order parameter *S* in erythrocytes of healthy donors were noticed (Fig. 3). Changes in membrane fluidity were connected with changes in concentration of cholesterol in erythrocytes.

In vivo studies (on animal and on human) showed that flavonoids have antidiabetic, antihyperlipidemic and antioxidant properties which are associated with their ability to decrease blood glucose level, decrease plasma total cholesterol concentration and enhance antioxidant system [67, 69–72].

In conclusion, our results suggest that flavonoids supplementation in dyslipidemic patients has a beneficial effect on the cholesterol concentration in erythrocytes membrane and on membrane fluidity. The present observations suggest that quercetin and cyanidin 3-glucose have more influence on concentration of cholesterol in erythrocytes membranes than hydroxycinnamic acids. Erythrocytes, as reported in this work, could be used as an experimental model to evaluate the beneficial effects of the flavonoids on plasma membrane cells.

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