In vitro effects of resveratrol on oxidative stress in diabetic platelets

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Abstract To evaluate the in vitro effects of resveratrol (RSV) incubation on platelets from compensated and decompensated diabetic patients in order to use it as an adjuvant therapy. The study was performed on 77 diabetic patients and divided into two phases: 29 compensated and 48 decompensated diabetic platelets were analyzed at recruitment (T0) and after in vitro RSV incubation (20 µg/ml) for 3 h at 37 °C (T1). Lipoperoxide and nitric oxide (NO) levels, superoxide dismutase (SOD) and Na+/K+ ATPase activities, total antioxidant capacity (TAC), and membrane fluidity tested by anisotropy of fluorescent probes TMA-DPH and DPH were determined. In vitro RSV incubation counteracts oxidative damage associated with diabetes and its complications; it is able to improve platelet function through augmented membrane fluidity and Na+/K+ ATPase activity; it enhances antioxidant systems’ functionality by increasing NO levels, SOD activity, and TAC and by decreasing lipoperoxide levels in both compensated and decompensated patients. Such platelet functionality enhancement suggests a new method of secondary prevention of complications associated with platelet dysfunction. Being free from one of the major risks associated with many antidiabetic agents, it can be assumed that RSV utilization in the diabetic diet may have a preventive and protective role in the progression of diabetic oxidative damage.

Keywords Lipoperoxide · Superoxide dismutase · Total antioxidant capacity · Nitric oxide · Membrane fluidity · Na+/K+ ATPase

Introduction

Diabetes mellitus (DM) is one of the most common metabolic diseases in the world: its incidence and prevalence are growing, especially in developing countries. The diabetic onset is preceded by a long time of chronic hyperglycemia that increases mitochondrial production of oxygen and nitrogen reactive species. In physiological conditions, this production is balanced by cellular antioxidant pools which are able to preserve the oxidation–reduction homeostasis; when this balance ends, a condition of oxidative stress is established [1]. Moreover, the pancreatic β-cells have a reduced antioxidant pool, especially the enzymatic one, so they are more exposed to oxidative stress damages [2].

Many studies have demonstrated that oxidative stress is engaged with the pathogenesis of diabetes, specifically with insulin resistance, insulin secretion progressive deficiency, and chronic complications’ onset [3, 4].

The considerable socioeconomic impact of DM justifies the interest of scientific research toward new molecules able to act on the various pathogenic mechanisms of disease, considering that lifestyle and nutritional interventions are essential. To date, numerous studies have been focused...
on the use of dietary antioxidants and nutraceuticals in the prevention of diabetic complications [5].

In recent years, resveratrol (RSV) molecule has received considerable attention for its potential benefits in human health [6].

Resveratrol (3,5,4-trihydroxystilbene) is a polyphenol found in grapes (Vitis vinifera, L.), a variety of berries, peanuts, and medicinal plants, such as Japanese knotweed (Polygonum cuspidatum, L.) [7]. The most important dietary source of RSV1 is red wine, and it is often postulated to be an important factor in the French paradox, a term coined to describe the very low incidence of cardiovascular disease in French population, despite a diet high in saturated fats [8].

Resveratrol has been reported to exert a variety of pharmacological effects. Studies using purified enzymes, cultured cells, and laboratory animals have suggested that RSV has antiaging, anticarcinogenic, anti-inflammatory, and antioxidant properties that might be relevant to prevent or slow the progression of a variety of conditions, including cancers, cardiovascular diseases, and ischemic injuries, as well as enhance stress resistance and extend life span in humans [9].

Resveratrol was also proved to possess an insulin-like effect and to promote the glucose uptake by hepatocytes, adipocytes, and skeletal muscle and hepatic glycogen synthesis in streptozotocin-induced diabetic rats [10].

Fully defining the targets of RSV that are biologically relevant is an enormous task, and it is made more difficult by the questions of whether these effects are either direct or indirect, and often conflicting results are reported in different systems.

Thus, based on the evidences reported so far, the aim of the present study was to evaluate the in vitro effects of 3 h RSV incubation (final concentration of 20 μg/ml) at 37 °C on platelets from subjects affected by DM with a good (DMc) and a poor glycometabolic control (DMde), in order to use it as a daily adjuvant therapy. Before (T0) and after (T1) such incubation, lipoperoxide levels by thiobarbituric acid–reactive substances (TBARs) assay, superoxide dismutase (SOD) and Na+/K+ ATPase activity, total antioxidant capacity (TAC), nitric oxide (NO) levels, and membrane fluidity by anisotropy of fluorescent probes DPH and TMA-DPH were measured.

Patients and methods

The study was performed on 77 patients affected by type 1 and type 2 DM (mean age 56 ± 15 years) consecutively admitted to the Department of Endocrinology and Metabolic Diseases of Ospedali Riuniti of Ancona between May and October 2011.

At recruitment, patients were divided into two groups:

- 29 subjects (mean age 54 ± 13 years) were in a good glycometabolic control (HbA1c < 7.0 %) (DMc)
- 48 subjects (mean age 57 ± 16 years) were in a poor glycometabolic control (HbA1c > 7.0 %) (DMde)

The average HbA1c was 7.8 ± 2 % while the average HbA1c value in DMc was 6.4 ± 0.5 %, and the average HbA1c value in DMde was 8.7 ± 1.3 %.

Oral hypoglycemic agent therapy was performed in all patients with type 2 DM, while insulin therapy was performed in all patients with type 1 DM. At enrollment, none of the patients had cancer, chronic liver disease, chronic renal failure, cardiovascular and cerebrovascular diseases, as well as any secondary form of diabetes. Nobody also reported episodes of hypoglycemia or has taken immunosuppressive agents. All patients were non-smokers and non-alcoholists and followed an unrestricted diet.

The study was performed in accordance with the principles contained in the Declaration of Helsinki as revised in 2001, and it was approved by the Review Board of Marche Polytechnic University. A written informed consent was subscribed by all subjects enrolled in the study.

Platelet isolation

After an overnight fast, peripheral venous blood was withdrawn from each subject and immediately mixed with anticoagulant citrate dextrose (ACD: 36 ml citric acid, 5 mmol/l KCl, 90 mmol/l NaCl, 5 mmol/L1 glucose, and 10 mmol/l ethylenediamine tetraceta, pH 6.8). Platelets were isolated by differential centrifugation in antiaggregation buffer (Tris–HCl 10 mmol/l, NaCl 150 mmol/l, EDTA 1 mmol/l, glucose 5 mmol/l, pH 7.4) [11]. This method involved a preliminary centrifugation at 200×g for 10 min to obtain platelet-rich plasma (PRP). PRP was then washed three times with antiaggregation buffer and centrifuged as above to remove any residual erythrocytes. A final centrifugation at 2,000×g for 20 min was performed to isolate the platelets. Platelets, obtained from DMc and DMde, were divided into two aliquots: one remained as it was (T0), while the other was incubated with RSV to a final concentration of 20 μg/ml for 3 h at 37°C (T1).

On platelets at both T0 and T1, SOD and Na+/K+ ATPase activities, membrane fluidity by anisotropy of fluorescent probes TMA-DPH and DPH, TBARs and NO levels, and TAC were assessed.

Lipoperoxide levels (TBARs)

Lipoperoxide levels were evaluated by Cayman’s thiobarbituric acid reactive substances (TBARs) assay Kit (N. 10009055); the measurement of these TBARs is a
well-established method for screening and monitoring lipid peroxidation.

Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds, such as malondialdehyde (MDA). MDA can be quantified following its controlled reaction with thiobarbituric acid (TBA); the MDA-TBA adduct formed by the reaction under high temperature (90–100 °C) and acidic conditions is measured colorimetrically at 530–540 nm.

For the standards, absorbance values (at 530 nm) on the Y axis versus MDA concentration (μM) in the X axis were plotted and the slope was calculated. MDA values for each sample was calculated from this standard curve according to the formula:

\[
\text{MDA (μM)} = \left[ \frac{\text{(corrected absorbance)} - \text{(y-intercept)}}{\text{slope}} \right]
\]

where the corrected absorbance is obtained by subtracting absorbance value of the standard A (0 μM) from itself and all other values of standards and samples.

Superoxide dismutase activity

Superoxide dismutase activity was evaluated by Assay Designs Stressgen colorimetric assay kit. Superoxide anion, generated from the conversion of xanthine and oxygen to uric acid and hydrogen peroxide by xanthine oxidase, converts WST-1 (water-soluble tetrazolium salts) to WST-1 formazan, a purple-colored product that absorbs light at 450 nm. SOD reduces the superoxide ion concentration and thereby lowers the rate of WST-1 formazan formation. SOD activity of the experimental sample is determined from percentage inhibition of the rate of formation of WST-1 formazan.

Absorbance values on the Y axis versus time in minutes, in the X axis, were plotted, and the slopes were calculated for each curve. Therefore, the percentage inhibition of the change in absorbance read at 450 nm was determined according to the formula:

\[
\% \text{ Inhibition} = \left( \frac{\text{slope activity control} - \text{slope of the sample}}{\text{slope control activity}} \right) \times 100
\]

Logarithms obtained from cell extracts of each sample were calculated, and the protein quantities that cause 50 % inhibition were determined; finally, SOD activity was calculated and expressed in units/μl.

Total antioxidant capacity (TAC)

Total antioxidant capacity was evaluated by Biovision Assay kit (total antioxidant capacity assay Kit, K274-100). Briefly, the Cu+ ion, reduced by antioxidants present in the sample, is chelated with a colorimetric probe giving a broad absorbance peak at around 570 nm, proportional to the total antioxidant capacity.

The final results were calculated using the following formula: \( S_a/S_v \), where \( S_a \) is the sample amount (nmol) extrapolated from the standard curve and \( S_v \) is the volume of undiluted sample added to the plate. The results were expressed as nmol/μl or mM Trolox equivalents.

NO levels

Nitric oxide production was evaluated by Assay Designs Total Nitric Oxide Assay Kit (Catalog No. 917-020; 192 Determination Kit). The kit involves the enzymatic conversion of nitrate to nitrite, by the enzyme nitrate reductase, followed by the colorimetric detection of nitrite as a colored azo dye product by Griess reaction, adding sulfanilamide (Griess reagent I) and \( N(1\)-naphthyl)ethylenediamine (Griess reagent II) [12–16], that absorbs visible light at 540 nm. The NO amount in each sample was determined using a standard curve generated with known concentrations of NO and expressed as nmol NO/mg protein. Protein concentration was determined by Bradford BioRad protein assay, using serum albumin as a standard to normalize the NO concentration data [17].

Membrane fluidity

Platelet plasma membrane fluidity was studied by determining the fluorescence anisotropy of probes 1-(4-trimethylamino-phenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH), which is incorporated in the lipid–water interface of the membrane bilayer, and 1-6-phenyl-1,3,5-hexatriene (DPH), which is a totally hydrophilic fluorescent probe [18]. Membrane incubation with TMA-DPH and DPH was performed as previously described [19].

The contribution of the light scattering was negligible in our samples because of the low cell concentrations used in the study as previously observed [20].

Steady-state fluorescence anisotropy \( (r) \) of TMA-DPH and DPH was calculated using the following equation:

\[
r = \left( \frac{I_v G - I_h}{I_v + 2I_h} \right)
\]

where G is the instrumental factor that corrects the \( r \) value for an unequal detection of vertically (Iv) and horizontally (Ih) polarized light. Fluorescence anisotropy is a quantitative index of the freedom of rotation of the probe; a decrease in the \( r \) value indicates a higher mobility of probes in the site where it is located, that is, increased membrane fluidity.

\( \text{Na}^+\text{K}^+ \) ATPase activity

\( \text{Na}^+\text{K}^+ \)-activated Mg\(^{2+} \)-dependent ATPase activity was determined in platelet plasma membranes according to the
Kitao and Hattori method [21]. The difference in inorganic phosphate (Pi) hydrolyzed in the presence and in the absence of ouabain was measured [22]. Protein concentration was determined with the Bradford BioRad protein assay using serum albumin as a standard [17], and Na\(^+/\)K\(^+\) ATPase activity was expressed as \(\mu\)mol Pi/mg prot/h.

Statistical analysis

Statistical analysis was performed using the SAS statistical package (Statistical Analysis System Institute, Cary, NC). Results are expressed as mean ± SD. ANOVA was used to analyze the difference in results obtained in different experimental conditions followed by Bonferroni t multiple-comparison test to reduce the probability of significant differences arising by chance. Differences were considered significant with \(p < 0.05\).

Results

In the present study 77 diabetic patients were analyzed and divided into two groups: DMc consists of 29 patients in good glycometabolic control, and DMde consists of 48 patients in poor glycometabolic control. General clinical parameters, provided by internal laboratory of the Division of Endocrinology, where the subjects were enrolled, are shown in Table 1. DMc and DMde patients showed statistically significant differences only for fasting glucose (131 ± 25 vs 198 ± 53 mg/dl, respectively; \(p < 0.05\)) and HbA1c (6.4 ± 0.5 vs 8.7 ± 1.3 %, respectively; \(p < 0.05\)).

Platelet lipid peroxidation, measured by TBARs assay, showed a statistically significant decrease at \(T_1\) compared to \(T_0\) \((p < 0.05)\) in both DMc and DMde patients, after RSV incubation at 20 \(\mu\)g/ml. Furthermore, significant lower levels in DMc compared to DMde patients at \(T_0\) as well as at \(T_1\) have been observed \((p < 0.05)\) (Fig. 1).

Regarding SOD activity and TAC, a statistically significant increase at \(T_1\) compared to \(T_0\) \((p < 0.05)\), in both DMc and DMde patients, has been observed, after RSV incubation at 20 \(\mu\)g/ml. Moreover, significant higher levels in DMc compared to DMde patients at \(T_0\) as well as at \(T_1\) have been observed \((p < 0.05)\) (Figs. 2, 3).

The same trend was found for NO levels: They significantly increased at \(T_1\) compared to \(T_0\) \((p < 0.05)\) in DMc and DMde patients, after RSV incubation at 20 \(\mu\)g/ml. Significant higher levels in DMc compared to DMde patients at \(T_0\) as well as at \(T_1\) have been shown \((p < 0.05)\) (Fig. 4).

### Table 1 Clinical parameters of compensated (DMc) and decompensated (DMde) diabetic patients

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Compensated patients (DMc)</th>
<th>Decompensated patients (DMde)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>29</td>
<td>48</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54 ± 13</td>
<td>57 ± 16</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>16/13</td>
<td>19/29</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>131 ± 25</td>
<td>198 ± 53*</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>6.4 ± 0.5</td>
<td>8.7 ± 1.3*</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>188 ± 42</td>
<td>179 ± 40</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>48 ± 14</td>
<td>46 ± 12</td>
</tr>
<tr>
<td>Total Chol/HDL Chol</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>115 ± 36</td>
<td>105 ± 35</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>131 ± 55</td>
<td>142 ± 75</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD

* \(p < 0.05\)
The anisotropy of TMA-DPH and DPH fluorescent probes, which indicates, respectively, the outer and the inner layer fluidity of platelet membrane, showed a statistically significant decrease in both DMc and DMde patients at $T_1$ compared to $T_0$ ($p < 0.05$), indicating an increase, respectively, in the outer and the inner membrane fluidity, after RSV incubation at 20 μg/ml. Furthermore, at both $T_0$ and $T_1$, the enzyme activity was significantly higher in DMc compared to DMde patients ($p < 0.05$) (Fig. 7).

**Discussion**

Studies about onset and progression of diabetes, as well as the pathophysiology of its secondary complications are increasingly focused on the role of oxidative stress, although the specific mechanisms have not yet been clarified [3, 23–25]. In diabetes, characterized by chronic hyperglycemia, a condition of oxidative stress is established, due to glucose autoxidation, which leads to an
excessive reactive oxygen species (ROS) production, decreased antioxidant defenses, and reduced effectiveness of the enzymes responsible for free radical elimination [26–28].

Specifically, in pancreatic β-cells, being poor of enzymatic antioxidant defenses (catalase, glutathione peroxidase, and superoxide dismutase), the oxidative stress acts directly by causing apoptosis and indirectly by activating stress-inducible cellular pathways; this activation may contribute, together with other pathogenetic mechanisms (advanced glycation protein product formation and lipid peroxidation), not only to complication appearance but also to insulin resistance and deficient insulin secretion onset [29].

In addition, chronic hyperglycemia affects platelet function, directly by activating protein kinase C (activation effector of platelet) and indirectly through the glycation of proteins by reducing the fluidity of the membrane, an essential property in the hemostatic process [4]. Hyperglycemia, inducing oxidative stress, alters endothelial function and the production of NO and prostacyclin I₂ (PGI₂), and it also contributes to the β-cell functional deficit, responsible for the progressive reduction in insulin secretion. Insulin works by inhibiting platelet activation so that the insulin deficiency, absolute or relative, appears to be an additional pathogenic factor responsible for the increased platelet adhesiveness and aggregation typical of diabetes, and thus promoting the formation of atherosclerotic plaques [30].

Recently, strategies for the prevention of diabetes and its chronic complications have focused on the reduction in oxidative stress through the use of dietary antioxidants and nutraceuticals [31].

Resveratrol is an excellent nutraceutical, and in the past decade, it has received widespread attention as either a potential therapy or a preventive agent for numerous diseases [6]. RSV plays its health beneficial effects on its anti-inflammatory, antioxidant, and anticarcinogenic properties, through multiple mechanisms of action [7]. RSV further interacts with a large number of receptors, kinases, and other enzymes that could plausibly make major contributions to its biological effects [6].

Moreover, RSV, through its antioxidant properties, is able to decrease intracellular ROS production, both by a direct scavenging and by inducing antioxidant enzymes’ expression, leading to an overall reduction in oxidative stress [32]. These antioxidant effects of RSV are probably related to the presence of hydroxyl groups that are able to trap the ROS on the skeleton stilbenic [33].

In addition, in rodent models of diet-induced obesity, RSV is able to improve insulin sensitivity and reduce body weight [34], which has led to much speculation about its potential as an antidiabetic in humans.

A recent study on streptozotocin-induced diabetic rats has shown that a 30-day RSV treatment induced a decrease in oxidative stress, characteristic of the diabetic state, by decreasing TBAR levels and increasing SOD and catalase activity; this suggests that RSV may have a protective effect against the liver and renal damage induced by oxidative stress in the diabetic state, which has been evidenced by the polyphenol ability to modulate the antioxidant defense and to decrease the lipid peroxidation in these tissues [35].

Another study, conducted on lipopolysaccharide/D-galactosamine rats, has shown that RSV treatment attenuates liver damage by decreasing lipid peroxidation measured by TBARS and conjugated dienes and increasing glutathione peroxidase and catalase activity [36].

In line with the research conducted to date, our study showed that in vitro RSV incubation induces a significant decrease in oxidative stress on DMc and DMde platelets; specifically, a decrease in lipoperoxide levels, as well as an increase in total antioxidant capacity, SOD activity, and NO levels has been observed. In addition, before and after RSV incubation, higher levels of SOD activity and TAC have been observed in DMc compared to DMde patients. These defense mechanisms, able to counteract the peroxidation, are more effective in DMc compared to DMde patients, since DMde
patients should also counteract the effects of failed glyco-
metabolic control. In addition, DMc patients, showing lower blood glucose levels than DMde ones, exhibit a lower non-enzymatic glycation of SOD NH$_2$ residues. It is well known that this glycation process may potentially deeply alter the structure, and then the function of proteins being directly proportional to the blood glucose concentration.

An additional mechanism involved in beneficial effects of RSV is the ability to maintain sufficient NO bioavail-
ability in the vascular endothelium [33]. Different studies have demonstrated that, at pharmacological doses, RSV increases vascular NO levels and improves NO bioavail-
ability in animal models [37, 38]. In vitro studies have been conducted to determine whether RSV acts directly on blood vessels or endothelial cells, facilitating NO produc-
tion; RSV did not affect eNOS activity, but instead inhibited NADH/NADPH oxidase and the subsequent decrease in superoxide generation, leading to an improved NO bioavailability [9].

In accordance with the literature, our study showed increased NO levels after RSV incubation in platelets of both DMc and DMde patients. In addition, NO levels were significantly higher, both at $T_0$ and at $T_1$, in DMc compared to DMde patients. This could be explained by a higher non-
 enzymatic glycation of nitric oxide synthase (NOS) NH$_2$ residues of DMde with respect to DMc patients, on the one hand, and by a higher intracellular ROS production through the polyol pathway stimulated by glucose excess, on the other hand. ROS may potentially alter the structure of any molecule (carbohydrates, lipids, proteins, and nucleic acids), and in particular, the intracellular ROS may alter the structure of NOS enzyme that is responsible for NO synthesis.

Recent studies on fluorescence and anisotropy [39] indicate that RSV has a fluidifying effect on the membrane and it is able to permeate also in the gel phase. Specifically, RSV acts as a spacer causing fluidification and disordering of the lipid molecules in the membrane [39]. These results reflect the well-described antioxidant effect of RSV, since antioxidants have to reach the rigid peroxidized mem-
branes and increase membrane fluidity in order to more efficiently interact with the lipid radicals in the disordered lipid bilayer. RSV also has a distribution within the membrane which is favorable for scavenging of lipid radicals; it has been clarified with probes positioned at different depths of the membrane, suggesting that RSV penetrates into the acyl region but also it places its hydroxyl polar group near the polar head region of the membrane.

Accordingly, our study showed, after in vitro RSV incubation, a significant decrease in TMA-DPH and DPH anisotropies in DMc and DMde diabetic platelets; being anisotropy inversely proportional to membrane fluidity, the observed decrease in anisotropy means that RSV incubation induces an increase in platelet membrane fluidity, both in the outer and in the inner layer. Specifically, TMA-DPH and DPH anisotropies, both before and after RSV incubation, in DMc compared to DMde patients, showed lower values, indicating an increased fluidity both in the external and in the internal layer; this could be explained by a minor glycosylation extent and minor plasma lipoprotein profile alteration, due to a lower blood glucose concentration in DMc patients [40].

Furthermore, at $T_1$ compared to $T_0$, a significant increase in Na$^+$/K$^+$ ATPase activity has been also observed in DMc and DMde platelets. This increased Na$^+$/K$^+$ ATPase activity observed after RSV incubation can be correlated to the increase in platelet membrane fluidity in the same subjects. Moreover, at both $T_1$ and $T_0$, Na$^+$/K$^+$ ATPase activity showed higher levels in DMc than in DMde patients; this effect, as observed for SOD activity, can be explained by a lower non-enzymatic glycation of Na$^+$/K$^+$ ATPase NH$_2$ residues and by a lower intracellular ROS production, both due to a lower blood glucose in DMc patients. A recent study has already shown that a 7-day incubation with RSV in Wistar rats, with an induced transient global cerebral ischemia, may decrease ROS production and lipid peroxidation and is able to retrieve Na$^+$/K$^+$ ATPase activity to normal levels [41]. Na$^+$/K$^+$ ATPase activity has been studied since this enzyme plays a key role in regulating cellular homeostasis through the maintenance of Na$^+$ and K$^+$ transmembrane gradient [42], but also indirectly of Ca$^{++}$ transmembrane gradient, involved in the activation and cellular response to platelet aggregating stimuli. Na$^+$/K$^+$ ATPase is also a marker of membrane function because it is a transmembrane protein that depends on its operation from the physicochemical properties and composition of the microenvironment where it is embedded [43]. Our study shows, in fact, an increased Na$^+$/K$^+$ ATPase activity in diabetic platelets incubated with RSV, being this enzyme located in a more fluid microenvironment due to a greater protection from oxidative damage and intrinsic properties of RSV.

Several studies show altered platelet functions in dia-
abetes and, in particular, decrease in membrane fluidity and Na$^+$/K$^+$ ATPase activity [37, 44]; therefore, an increased membrane fluidity and Na$^+$/K$^+$ ATPase activity, as observed in our study, highlight an improvement in platelet function, which in turn could slow the progression of dia-
abetic complications.

Considering all the results obtained in this study, it could be proposed that RSV incubation contrasts oxidative damage associated with diabetes and its complications, by improving platelet function through increased membrane fluidity and Na$^+$/K$^+$ ATPase activity, on the one hand, and
by enhancing antioxidant systems’ functionality through increased SOD activity, TAC and NO levels, and decreased lipoperoxide levels, on the other.

Such platelet function improvement, which results in hyperactivity and hyperaggregability reduction, characteristics of DM, suggests a new approach of secondary prevention for platelet dysfunction–associated complications. Moreover, since from very early stages of diabetes natural history, oxidative stress plays a role in causing chronic complications, RSV supplementation could be proposed even in primary prevention, after an in vivo thorough testing. RSV supplementation, as an adjunct to insulin therapy in diabetic patients, is related to its easy availability, safety, low cost, and lack of side effects, unlike many antidiabetic agents.

Given that symptoms of hypoglycemia have not been reported yet, it appears that RSV is likely to be free from the major risks associated with many antidiabetic agents, making it an attractive choice if efficacy can be more thoroughly demonstrated.

In conclusion, it could be assumed that the RSV use in diabetic diet may have first a preventive then a protective effect in oxidative damage progression associated with DM and its complications, although the exact protective mechanism is still under study.

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Conflict of interest None.

References


