Berries and oxidative stress markers: an overview of human intervention studies

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Berries are an excellent source of bioactive compounds such as vitamins, minerals but above all polyphenols with anthocyanins as the most representative compounds. Several in vitro and in vivo studies documented the beneficial effects of berries and their bioactives in the modulation of numerous cell functions related to oxidative stress and/or antioxidant protection.

The following review summarizes published results about the role of berries (either fresh, juice, freeze-dried or dehydrated) on total plasma and serum antioxidant status and on the modulation of biomarkers of oxidative stress in acute and chronic human intervention trials. The biomarkers considered include DNA, protein and lipid oxidation, and endogenous antioxidant enzymes.

Though limited, there is indication that the consumption of berries may reduce oxidative stress by modulating protein and lipid oxidation, and by improving total antioxidant status. In particular, these effects are more evident following medium/long term interventions with respect to postprandial studies. Benefits are observed in healthy subjects as well as in those with cardiovascular risk factors or other diseases. On the contrary, data regarding the effect of berries on DNA damage and endogenous antioxidant enzyme activities are still scarce and inconclusive. In conclusion, much remains to be elucidated before a comprehensive understanding of the effects of berries on the modulation of oxidative stress markers is achieved. Robust clinical evidence supporting the role of berries in counteracting oxidative stress in humans is encouraged.
INTRODUCTION

In the last decade, polyphenol-rich foods have received increased interest from researchers and the food industry. The main reason for this interest is the recognition of their potential protective effects in human health and disease prevention. Berries are a rich source of polyphenol-bioactives and recent in vitro and in vivo evidence seems to support their role in the prevention of various diseases associated with oxidative stress.1-8 Furthermore, they positively affect plasma antioxidant status in humans and they are involved in the modulation of several physiological functions and in the activity of a wide range of endogenous enzymes.2-9-11 In the present review, we attempt to summarize the main literature on the role of berry consumption on antioxidant status and on the modulation of oxidative stress markers in humans. In particular, we evaluated markers of DNA, protein and lipid damage, and modulation of endogenous antioxidant enzymes following acute and chronic dietary interventions with berries. Moreover, a short overview on absorption and metabolism of the main berry bioactives and a critical analysis of the studies and their results was included.

Berry Fruits, Bioactives and Bioavailability

Among berries, it is necessary to point out that the term “soft fruits” refers to a wide number of berries, mainly belonging to the genera Vaccinium and Rubus. The most common berries include: highbush blueberry (Vaccinium corymbosum), lowbush blueberry (Vaccinium angustifolium), bilberry (Vaccinium myrtillus), cranberry (Vaccinium macrocarpon), blackberry (Rubus fruticosus), black raspberry (Rubus occidentalis), red raspberry (Rubus idaeus), blackcurrant (Ribes nigrum), strawberry (Fragaria ananassa), lingonberry (Vaccinium vitis-idaea), cloudberry (Rubus chamaemorus), elderberry (Sambucus nigra L.), and chokeberry (Aronia melanocarpa). Berries can be consumed as fresh fruits as well as ingredients in many processed products including yogurts, purées, juices and jams. Moreover, a raising trend in using berry extracts as ingredients in numerous
dietary supplements has been documented in the last years. In addition, the food industry has pushed toward the use of berries and berry products as functional foods.

Most berries contain high levels of phenolic compounds including flavonoids (anthocyanins, flavonols and flavanols), condensed tannins (proanthocyanidins), hydrolyzable tannins (ellagitannins and gallotannins), phenolic acids (hydroxybenzoic and hydroxycinnamic acids, chlorogenic acid), stilbenoids and lignans. These compounds are well recognized for their antioxidant activity, which may play a crucial role in the prevention of many chronic diseases.

The concentration of phenolic compounds varies according to species, genotype, growing and post-harvesting conditions of berries. The most characteristic group of phenolics in berries is probably the class of anthocyanins (ACNs). More than 500 different ACNs have been described, and six of them (i.e. pelargonidin, cyanidin, delphinidin, petunidin, peonidin, and malvidin) are commonly found in berries. Usually, their content increases during ripening and can reach values up to 4-5 g/kg fresh weight (FW) in blackcurrants, black elderberries, blackberries and blueberries, and up to 0.6-0.8 g/kg FW in strawberries. ACNs are mainly found in the skin but can also appear in the flesh (e.g. strawberries). It is estimated that the average total intake of ACNs may be approximately 200 mg/day; however, they are generally poorly absorbed with less than 1% of the ingested amount reaching the plasma where the concentration ranges between 10 and 50 nmol L⁻¹.

Bioavailability differs for the type of berry and the process. Recently, it has been shown that the availability of ACNs from berry juice is lower than that from whole berries. Kuntz and colleagues reported that the absorption of Mv-3-glc after juice intake was lower than that after smoothie ingestion, indicating that malvidin-3-glucoside (Mv-3-glc) from juice was more available (80% relative bioavailability). Del Bo’ and co-workers documented that blanching increased the absorption of ACNs in a blueberry purée compared to an unblanched product. Additionally, the bioavailability of ACNs varies markedly depending on the food matrix, including other antioxidants, micronutrients, and macronutrients present in the foods consumed.
colleagues investigated the occurrence of ACNs and phenolic acids (PA) in urine after ingestion of fruit purée with and without oat cereals. They observed a delayed maximum urinary excretion of dietary PA after consumption of purée and cereal compared with consumption of purée alone. Additionally, Cebeci et al. documented that whole or skimmed milk did not affect the total phenolic content and the bioavailability of phenolic compounds.

After absorption, ACNs are rapidly metabolized to glucuronidated, methylated and sulfated compounds in the liver. Some studies reported that the urinary concentration of glucuronides and methylated glucuronide forms is four to six times higher than the native ACNs. In fact, glucuronides and methylated compounds are the predominant forms detected in plasma and urine, while very few studies identified sulphated of the cyanidin-3-glucoside (Cy-3-glc) and Mv-3-glc. Most ACNs reach the colon where they are rapidly broken down by the microbiota into phenolic degradation products. These compounds can be absorbed and undergo additional metabolic transformation. These processes may affect the biological activity of the newly constituted compounds.

Gallic (GA) and chlorogenic acids (CGA) are the most abundant phenolic compounds in blueberry (up to 2 g/kg FW in particular CGA) and blackberries (about 0.3 g/kg FW), while in strawberries the concentration is low (about 0.09 g/kg FW). GA and conjugates are rapidly absorbed and generally, they reach a maximum concentration between 1 and 2 h in plasma following the consumption of berries. On the contrary, CGA is poorly absorbed; only one-third of ingested amount is absorbed in the small intestine, while the remainder is largely transformed into caffeic acid and/or metabolites.

Berries are also an excellent source of flavan-3-ols, a complex subclass of polyphenols without glycosidic residues and with different levels of polymerization including monomers, oligomeric and polymeric proanthocyanidins. These latter compounds can be classified according to their monomeric units (i.e. monomers of epicatechins are named procyanidins) or the position of...
carbon-carbon or carbon-oxygen intermolecular bonds. Chokeberries, blueberries and strawberries are a rich source of flavan-3-ols and proanthocyanidins (from 1.5 up to 6.6 g/kg FW), while blackberries and raspberries contain around 0.3 g/kg FW. The bioavailability of these compounds may vary greatly according to their molecular weight and the food matrix. Studies reported that about 8–17% of dietary flavan-3-ols are absorbed in the small intestine, while the unabsorbed fraction reaches the colon where it is transformed into several low molecular weight metabolites (i.e. phenylpropionic, phenylacetic, hippuric, benzoic acids).

Hydrolysable tannins are the main group of plant tannins including more than 500 compounds composed of sugar polyesters (usually glucose) and phenolic acids. Berries are an important source of ellagitannins particularly abundant in cloudberry and red raspberry where they account for about 80% of total phenolics (up to 2.6 g/kg FW), and in blackberries, blueberries and strawberries forming 51% of the total compounds (up to 6 g/kg FW). After ingestion, tannins are hydrolyzed to ellagic acid; this compound can be absorbed and metabolized by phase II enzymes into sulfated, glucuronidated and methylated compounds, or by microbiota into urolithins.

Berries also contain vitamin C, provitamin A carotenoids, E, and B vitamins that can contribute to antioxidant protection. They are present in honeyberry and blackcurrants in concentration higher than those found in raspberries, gooseberries and strawberries. The level varies according to numerous factors including genetics, environmental and storage conditions. Vitamin C is reported in high amounts in blackcurrants (0.7–2.8 g/kg FW) and strawberries (about 0.6 g/kg FW).

Berries are also a source of minerals. Some minerals contained in berries such as copper, iron, zinc, manganese and selenium are important cofactors and components of antioxidant enzyme systems (e.g. superoxide dismutase, SOD; glutathione peroxidase, GSH-Px), contributing to antioxidant defense. The major mineral elements found in berries are phosphorus, potassium, calcium, magnesium, iron, manganese, copper, sodium, and aluminum. Blackcurrant, strawberry,
raspberry, blackberry and blueberry contain more calcium (150-350 mg/kg FW), potassium (0.5-3.2 g/kg FW) and manganese (12-39 mg/kg FW) compared to other berries.\textsuperscript{2,6,17,33}

**Antioxidant Defense, Oxidative Stress and Biomarkers**

Berry bioactive components exert an important role against oxidative insults by acting as scavengers for free radicals. Oxidative stress occurs following an imbalance between the cellular production of oxidant molecules and the availability of antioxidants able to defeat these insults.\textsuperscript{34} Reactive oxygen and nitrogen species (ROS/RNS) are the major contributors to the development of oxidative stress. They include superoxide anion (\(\text{O}_2^-\)), hydroxyl radical (\(\text{HO}^-\)), lipid radicals (\(\text{ROO}^-\)), nitric oxide (NO), hydrogen peroxide (\(\text{H}_2\text{O}_2\)), peroxynitrite (\(\text{ONOO}^-\)) and hypochlorous acid (\(\text{HOCl}\)).\textsuperscript{35} ROS production is involved in cell damage, necrosis and cell apoptosis due to the oxidation and nitration of cellular proteins, lipids and DNA, that bring loss of cell function.\textsuperscript{34} In order to measure oxidative stress conditions, several biomarkers and analytical methods have been developed. The most commonly exploited markers include the evaluation of antioxidant capacity in the bloodstream (serum/plasma), the estimation of antioxidant defense system (enzymes and endogenous compounds), and the evaluation of the levels of oxidative damage to DNA, proteins and lipids. The European Food Safety Authority (EFSA) stated that the protection of tissues, cells, and biomolecules (i.e. DNA, proteins, and lipids) is a beneficial physiological effect for humans; however, the substantiation of antioxidant protection requires target molecules in vivo and appropriate methods of assessment. In this regard, the utility and validity of biomarkers for oxidative stress are still under debate and main criticisms reported in the EFSA statements will be mentioned.

-**Total Antioxidant Capacity and Defense**

Antioxidants exert an important role against free radical damage; thus, measurement of antioxidant levels in biological fluids is used to assess the extent of oxidant exposure, and in turn, oxidative
stress. The evaluation of the antioxidant status represents one of the main approaches used in human studies to evaluate changes of the total antioxidant capacity (TAC) in a target tissue or biological fluids (i.e. plasma/serum) following, for example, a dietary treatment. Numerous methods have been developed and the most common include the total reactive antioxidant potential (TRAP), the trolox-equivalent antioxidant capacity (TEAC), the ferric reducing antioxidant potential (FRAP), the oxygen radical absorbance capacity (ORAC) or the ferrous oxidation-xylenol orange (FOX) assays. They differ from each other in terms of reaction mechanisms, oxidant and target/probe species, reaction condition and expression of the results obtained. Over the years, researchers discussed the validity of these methods emphasizing their numerous limitations. All of them provide an estimate of the antioxidant capacity of plasma/serum without distinguishing the contribution by exogenous molecules of dietary origin (i.e. ascorbic acid, vitamin E, polyphenols) compared to endogenously-derived molecules such as enzymatic components (i.e. SOD, GSH-Px, and catalase, CAT) and small macromolecules (i.e. albumin, bilirubin, ceruloplasmin, ferritin, glutathione). From a clinical perspective, this poses problems for data interpretation. In this regard, EFSA remarked the inappropriateness of the methods used to determine antioxidant capacity in humans and extrapolate possible effects on human health. Since results vary across different TAC assays, these methods should be assessed in parallel with others biomarkers of oxidative damage. SOD, CAT and GSH-Px are the most widely studied enzymes involved in oxidative stress. SOD is involved in the dismutation of superoxide into oxygen and H$_2$O$_2$ as part of the antioxidant defense system. GSH-Px is a general name of an enzyme family involved in removal of peroxide in the tissues. They utilize reduced glutathione as a substrate to convert peroxides and hydroperoxide into alcohols, water and oxidized glutathione. Lastly, CAT is a family of enzymes involved in the decomposition of H$_2$O$_2$ into water and oxygen. These enzymes work in conjunction
and therefore the measurement of all three together could be useful to determine the antioxidant status.$^{34}$

Glutathione S-transferases (GSTs) are a family of enzymes involved in the metabolism of xenobiotics and carcinogens thus they play an important role in the protection against oxidative stress.$^{34}$ GSTs bind and conjugate electrophiles to reduced glutathione neutralizing them and protecting the cell from deleterious effects. In addition, some GSTs also have glutathione peroxidase activity.$^{34}$

Regarding antioxidant enzymes, EFSA has stated that measurement of enzyme induction alone, is not sufficient as evidence for claims related to the “antioxidant defense system”.$^{37}$

-Oxidative Damage to DNA

Numerous studies have shown that oxidative DNA damage is associated to a variety of aging-associated degenerative diseases such as cancer and cardiovascular disease (CVD).$^{38}$ Genomic damage can be caused by a variety of physical and chemical agents such as ultraviolet and ionizing radiation, xenobiotics and endogenous ROS. The most common types of DNA damage include base loss, base deamination, base alkylation, base dimerization, base oxidation and single/double strand breakage.$^{39}$ Nuclear and mitochondrial DNA from tissue and blood lymphocytes can be used to evaluate oxidative damage. ROS formation may lead to oxidized DNA bases, apurinic/apyrimidinic sites or DNA strand breaks. Among all purine and pyridine bases, guanine is the most prone to oxidation and the most commonly oxidized base lesion is the 8-oxo-2'-deoxyguanosine (8-OHdG).$^{40}$ 8-oxodG is a biomarker reflecting the balance between oxidative damage and repair rate.$^{38}$ It is unstable, mutagenic and it can react with compounds such as peroxynitrite to even more mutagenic lesions. Urinary 8-OHdG has been measured as indicator of oxidative damage.$^{40}$ The methods used to estimate 8-OHdG include high performance liquid chromatography-electrochemical detection (HPLC-EC), gas chromatography–mass spectrometry
(GC-MS), LCMS/MS, antibody-based immunoassays, 32P-post-labelling and enzyme-linked immnosorbance assays (ELISA), direct enzymatic detection by using bacterial glycosylases and endonuclease enzymes.\textsuperscript{39,41}

The single cell gel electrophoresis (comet assay) represents a relatively simple technique that allows the measure of DNA strand breaks at the levels of individual cells. Direct measurements of oxidative damage to DNA could be obtained \textit{in vivo} by using modifications of the comet assay which technique allows the detection of oxidized DNA bases.\textsuperscript{37} This assay directly reflects DNA oxidative damage within cells when assessed, for example, in peripheral blood mononuclear cells (PBMCs). The main methods evaluating oxidized DNA bases involve specific enzymes that recognize oxidized bases such as endonuclease III (ENDO III) able to detect oxidized pyrimidine bases, and formamidopyrimidine (FPG) enzyme (FPG-sensitive sites) that acts on oxidized purines. In addition, the comet assay can be used to study DNA \textit{ex vivo} resistance to oxidative stress by incubating the cells with a stressor (e.g. H\textsubscript{2}O\textsubscript{2}, Fe\textsuperscript{3+}). Measurements of mRNAs of DNA repair enzymes (e.g. oxoguanosine glycosylase 1 and apurinic/apyrimidinic endonuclease 1) has been recently included in several dietary intervention studies, but the results are still inconclusive.\textsuperscript{42}

Considering the Consensus Statement published by EFSA neither of these measurements alone should be considered sufficiently powerful to assess \textit{in vivo} oxidative damage to DNA and demonstrate antioxidant protection.\textsuperscript{37} On the whole, oxidized DNA bases have been recognized as a reliable marker of oxidative damage while other markers may present greater limitations related to the impact of oxidative damage and repair process and to analytical and technical aspects.\textsuperscript{37}

\section*{-Oxidative Damage to Proteins}

Proteins represent the major target for biological oxidants as result of their abundance and for their reaction with many species. Reactions can occur with both the side chains and backbone, with the extent of attack at particular sites of the protein. In some cases, damage is limited to specific
residues, whereas with other species (e.g. hydroxyl radicals) damage is widespread and nonspecific.\textsuperscript{43}

Plasma protein carbonyl content in biological fluids is actually the most generally used marker of protein oxidation. Carbonyl groups (aldehydes and ketones) are produced on protein side chains during the oxidation process.\textsuperscript{44} Many assays are available for detection of protein carbonyls. The best approach to determine oxidative damage to proteins can be obtained by means of HPLC-MS.\textsuperscript{37}

Another marker of protein oxidation is the nitrotyrosine, a product of tyrosine nitration mediated by reactive nitrogen species such as peroxynitrite anion and nitrogen dioxide. Nitrotyrosine in biological fluids and tissues is increasingly being used as an indicator or marker of cell damage, inflammation as well as NO production.\textsuperscript{45} The current gold standard technique for the measurement of nitrotyrosine is MS/MS coupled with GC or HPLC as long as identification and separation of such molecule in plasma from other substances is successfully achieved.\textsuperscript{46} Other ways of quantifying protein nitration are immunocytochemical and immunohistochemical assays based on either monoclonal or polyclonal anti nitrotyrosine antibodies.\textsuperscript{43,47}

Regarding the evaluation of oxidative damage to proteins, EFSA has stated that a direct measurement \textit{in vivo} can be obtained by means of HPLC-MS. The use of conventional assays, such as colorimetric or ELISA methods may only be used in combination with at least one direct marker of oxidative damage to proteins \textit{in vivo} if assessed directly in blood or tissue.\textsuperscript{37}

\textbf{-Oxidative Damage to Lipids}

Lipids are susceptible targets of oxidation because of their molecular structure often abundant in reactive double bounds. Oxidized LDL, lipid hydroperoxide, malondialdehyde (MDA), conjugates dienes, and isoprostanes (F\textsubscript{2}-IsoP) are products of lipid peroxidation. Compared to others, F\textsubscript{2}-IsoP are chemically stable end-products and for this reason are the most well studied markers.\textsuperscript{43}
F₂-IsoPs are a series of prostaglandin F₂a-like compounds produced \textit{in vivo} by non-enzymatic peroxidation of arachidonic acid, esterified in phospholipids and then subsequently hydrolysed to their free acid form by the platelets activating factor acetylhydrolase.\textsuperscript{43} IsoPs are released from the cell membrane into circulation by phospholipases, and can be quantified in all human tissues and biological fluids, including plasma, urine, cerebrospinal and broncho-alveolar lavage fluid.\textsuperscript{48} Direct measurements of oxidative damage to lipids (i.e. lipid peroxidation) could be obtained \textit{in vivo} by measuring changes in F₂-IsoP in 24-h urine samples (i.e. better matrix than plasma for this measurement).\textsuperscript{37} High levels of IsoPs in plasma and urine samples have been shown to correlate with \textit{in vivo} oxidative stress in a number of animal and human studies.\textsuperscript{49} IsoPs are elevated in association with risk factors such as hyperhomocysteinemia, hypercholesterolaemia, diabetes mellitus, obesity, cigarette smoking, as well as atherosclerosis.\textsuperscript{50}

F₂-IsoP levels are also elevated in human atherosclerotic lesions compared with normal vascular tissue, and may participate in the actual pathogenesis of atherosclerosis through effects on vasoconstriction, platelet aggregation, and proliferation of vascular smooth muscle cells.\textsuperscript{49-50} F₂-IsoP can be measured using GC/MS, LC/MS, ELISA, and radioimmunoassay in plasma and urine samples\textsuperscript{51} even if mass spectrometric techniques still remain the gold standard techniques for IsoP quantification.\textsuperscript{37,50}

Oxidative damage to lipids can also be obtained \textit{in vivo} by measuring oxidized low density lipoprotein (LDL) particles. This may be evaluated in blood using immunological methods (i.e. antibodies) with appropriate specificity. Phosphatidylcholine hydroperoxidases measured in blood or tissue by HPLC is also an acceptable marker of lipid peroxidation.\textsuperscript{37}

Thiobarbituric acid reactive substances, MDA, high density lipoprotein (HDL)-associated paraoxonases, conjugated dienes, breath hydrocarbons, auto-antibodies against LDL particles and \textit{ex vivo} LDL resistance to oxidation have been proposed as markers of lipid damage.\textsuperscript{37}
On the whole, EFSA states that the measurement of F$_2$-IsoP in urine, oxidized-LDL and phosphatidylcholine hydroperoxidases in plasma, represent reliable *in vivo* markers of lipid peroxidation. Other markers could be used in combination, if appropriate techniques are applied for the analysis.\textsuperscript{37}

**METHODS**

A search for literature on intervention studies investigating the role of berries in the modulation of antioxidant defense and oxidative stress was carried out. Human *acute* and *chronic* intervention studies involving berries and reporting measurement of oxidative stress markers in cells, plasma, serum, erythrocytes and urine were selected. PUBMED and ScholarGoogle databases were searched to identify papers published later than January 1\textsuperscript{st} 2000.

The searches used the following terms and keywords alone and in combination: ‘humans’, ‘berry’, ‘oxidative stress’, ‘DNA damage’, ‘lipid damage’, ‘protein damage’ and ‘antioxidant capacity’. The selection of markers was carried out taking into consideration those generally reported in intervention trials. Particular attention has been devoted to markers of antioxidant protection and/or oxidative stress for which scientific guidance (reporting biological and methodological critical aspects) has been provided by EFSA\textsuperscript{37}.

Only English-language papers were selected but no other publication data restrictions were applied. Interventions conducted in both healthy and pathological subjects were included in the revision.

Fifty-seven scientific papers were obtained from the database searches and from the reference lists of the obtained papers.\textsuperscript{52-107} Based on a preliminary review of the abstracts, eight articles were excluded because studies were performed with a mix of fruit and vegetables in which berries were not the main food.\textsuperscript{58,63,67-68,71,77,83-84} Moreover, studies were excluded if the control/placebo food included soft fruits, thus the beneficial effect could not be attributed specifically to the berries (Figure 1). No other exclusion criteria was applied (e.g. study design).
Therefore, after exclusions, a total of forty-nine studies investigating effects of soft fruits on markers of oxidative stress was included in the review. These papers were published in more than twenty different journals and studies were conducted in sixteen different countries, but mainly USA, Canada, Germany and Italy. The results obtained are reported in Table 1 and 2 describing the type of food or supplement, the number of intervention days, the number of subjects and their characteristics, the dose/day of test food and the content of its main bioactives, the use of control/placebo food, the outcomes measured and the significant findings.

Role of Berries in the Modulation of Biomarkers of Antioxidant Defense and Oxidative Stress

The impact of berry consumption on biomarkers of oxidative stress has been investigated in several acute (Table 1) and chronic (Table 2) human intervention studies. Three out of 49 studies performed both the acute and chronic interventions, while thirty-six performed chronic dietary intervention studies. The main berries investigated were bilberries, lingonberries, blueberries, blackcurrants, blackberries, raspberries, cranberries, chokeberries, strawberries, boysenberries, elderberries and whortleberries. Berries were characterized for the content of phenolic compounds (i.e. phenolic acids and ACNs), vitamins and antioxidant activity. Eight studies (one postprandial and seven chronic interventions) did not provide information about the composition of berry bioactive compounds. The amount of bioactives administered, varied from study to study and it was based on the type of berry, the amount of food administered, and the analytical method used for the characterization of the berries. Thus, comparison among studies and extrapolation of information about dose-response effects appears difficult due to the different characteristics of the tested food and subjects recruited; thus no comparison among studies could be done. Thirty-one studies were performed in healthy subjects, six in individuals with metabolic syndrome, three studies in subjects with CVD risk.
factors, two studies in people with type 2 diabetes mellitus, one study in hypertensive individuals, one study in individuals with fatty liver diseases, and one in subjects with premalignant esophageal condition.

In Table 1 are reported the main results on biomarkers of oxidative stress following the postprandial intake of berries. Of the thirteen studies considered, nine used a single food for the intervention, while five studies tested the effect of a mix of berries. Berries were provided mainly in the form of purées, juices or beverages (i.e. obtained by suspending a lyophilized berry product in water), while two studies used the whole fresh food approach. The dose of berries varied from 80 to 1000 g for whole berries/purée, and from 240 to 500 mL for the juice/beverage. Regarding the experimental design, most of the studies were placebo-controlled and crossover. Three studies were performed without placebo.

The effect of berries in the modulation of plasma and serum antioxidant status was evaluated in nine studies through TEAC, TRAP and ORAC assay. Eight out of nine studies showed that the intake of a single portion of berries significantly increased serum/plasma antioxidant status compared to the control group as assessed by the methods previously reported. The effect of the modulation of endogenous antioxidant enzymes was investigated only in one study that documented a significant increase in SOD and GSH-Px activity in red blood cells following the consumption of cranberry juice. Thus, no conclusion can be drawn about the role of a single portion of berries in the modulation on such biomarkers.

The impact of berry consumption on DNA oxidative damage was evaluated only in two studies. The markers considered were endogenous DNA damage (evaluated as FPG-sensitive sites), DNA ex vivo resistance to H₂O₂-induced oxidative damage, DNA single strand breaks, and plasma concentration of 8-OHdG. Del Bo’ et al. reported that the consumption of a single blueberry purée portion (300 g) was able to decrease oxidatively-induced DNA damage in PBMCs in healthy subjects. On the contrary, no effect was observed for the levels of endogenous DNA damage
implying that long-term supplementation is required to induce modification and protection on purine DNA bases. Mathison et al. documented no effect on plasma levels of 8-OHdG following the consumption of a cranberry leaf extract beverage.

The effect of berry consumption on the levels of oxidative protein damage (evaluated as protein carbonyl or total thiol serum groups in the blood) was performed in three studies. Two of them documented no effect following the consumption of berries in a group of healthy male and of athletic females, while the other one showed a reduction in plasma protein carbonyl levels following the intake of the berry beverage in a group of cyclists. The difference in the results could be attributed to several factors such as the protocols used to perform exercise and to induce an oxidative stress condition to the muscles, the level of training, the different foods tested (blueberry smoothie with banana versus a mix of berries versus blueberry sorbet), the number and the sex of subjects enrolled (males versus females).

The effect of berry consumption on lipid oxidation and damage was evaluated in five studies. The main biomarkers included diene conjugates, ox-LDL and MDA plasma levels. Three studies reported a significant reduction in lipid damage following the consumption of a single portion of strawberries, blackcurrants, or a mix of berries and fruit juice; while no effect was observed in two studies.

Table 2 summarizes the main results related to the effect of chronic consumption of berries in the modulation of biomarkers of oxidative stress. A total of thirty-six studies was analyzed. Thirty tested the effect of a single berry, three used a mix of berries and six were performed with supplements and capsules. Berries were provided mainly in the form of juices and/or beverages, while in seven studies the whole fresh food was used. The dose of berries varied from 100 g to 500 g for whole berries/purée and from 240 mL to 1000 mL for the juice/beverage. The duration of most studies was 4-6 weeks. Eighteen studies used a parallel experimental design, seven
a crossover design, while fourteen were conducted without a control/placebo group, thus the experimental design was classified as “baseline and post-intervention.”

The evaluation of antioxidant status through TEAC, TRAP and ORAC assays was performed in fifteen out of thirty-six studies. Twelve studies reported a significant effect following berry consumption, while three did not show any effect. Considering the effect of berries in the modulation of endogenous antioxidant enzymes such as SOD, GST, GSH-Px, only eight studies were conducted. Three trials documented a significant increase in enzymes activity following the intervention, while the others reported no significant findings.

Twelve studies investigated the effects of berry consumption on the levels of DNA damage, evaluated as DNA strand breaks, endogenous DNA damage (e.g. FPG and EndoIII-sensitive sites), H$_2$O$_2$-induced DNA damage and DNA repair capacity, measured by comet assay in PBMCs, and 8-oxodG detected in urine. The evaluation of endogenous DNA damage following berry treatment was investigated in three studies. Only one study showed a significant reduction in the levels of oxidized purines (FPG-sensitive sites) following 6-week wild blueberry intake in subjects with CVD risk factors; one study reported a lack of protective effect on Endo III-sensitive sites, while one study observed a significant increase in the levels of FPG sensitive sites after 3-week blackcurrant intervention in a group of healthy subjects. The authors speculated that the increase of DNA damage in the blackcurrant juice group could be attributed to a possible adverse effect related to the amount of vitamin C introduced (140 mg/day dose) or to a prooxidant effect of polyphenols that chelate iron ions in the presence of vitamin C. However, the authors pointed out that treatment effects did not differ in this respect, and the increase in FPG sensitive sites was rather small.
DNA resistance to oxidative stress was investigated in five dietary intervention studies. Three studies documented an improvement in the protection against oxidative damage following 6-week intervention with wild blueberry in subjects with CVD risk factors, 4-week intervention with blueberry/apple juice, and 2-week intervention with strawberries in healthy subjects. On the contrary, no effect was observed after 2-week intervention with cranberry juice, and 4-week consumption of blueberry/apple juice in healthy volunteers. The discrepancy between the two studies performed with blueberry/apple juice by the same authors was attributed to the number of subjects enrolled. In the first pilot trial only eight subjects were recruited and the results documented a large inter-individual variation in the levels of DNA damage. On the other hand, the second trial involved a large group of individuals (168 healthy volunteers) and that was sufficient to demonstrate a significant protection against DNA oxidative insult.

Lastly, three studies evaluated the effect of berry intervention on the levels of DNA strand breaks. All the studies considered did not show a significant effect of berries in the modulation of this marker. No effect was also observed, by Riso and colleagues, for the mechanisms involved in DNA repair capacity following 6-week supplementation with a wild blueberry drink. Probably, a longer-term exposure to diet is required to affect DNA repair mechanisms.

The impact of berry intervention on the levels of 8-oxodG was evaluated in six studies. Four studies showed no effect following the intervention with berries, while only one reported a significant reduction in urinary levels of 8-oxodG after 30 days of strawberry consumption, and a reduction in urinary levels of 5-hydroxymethyl-2'-deoxyuridine (5-OHMU) following 6-week intervention with blueberries. Based on these results, further studies are necessary to elucidate the role of berries in the modulation of 8-oxodG.

Six studies investigated the effect of medium/long term berry intervention on the levels of oxidative protein damage evaluated as protein carbonyl, reduced-SH thiols or advanced oxidation.
protein products (AOPP) in the blood. Despite the limited evidence, all the results obtained have shown a protective effect of berries against protein oxidative damage.

The effect of berries in the modulation of lipid damage was investigated in twenty-eight studies. Nineteen studies documented a beneficial effect on lipid damage, while nine studies did not show any effect. The main markers included MDA, ox-LDL, diene conjugates, F$_2$-IsoP, lipid peroxidation and 4-hydroxynonenal (HNE). MDA was the only marker of lipid damage that revealed a significant reduction following berry intervention. Nine trials investigated the impact of berries on the levels of F$_2$-IsoP, lipid peroxidation and HNE. Although limited, the results seem to support the beneficial effects of berry intervention on those markers. On the contrary, results about the effect of berries on ox-LDL are still inconclusive; about half of the studies showed a reduction of ox-LDL levels, while the other half no effect. Regarding diene conjugates, no study documented a significant effect following the intake of berries. Further studies are necessary to elucidate the role of berries in the modulation of ox-LDL and HNE.

MARKERS OF OXIDATIVE STRESS NOT CONSIDERED IN THE REVIEW

Others markers directly or indirectly related to oxidative stress, analyzed in the papers reviewed but not discussed in the report, include markers of endothelial function (i.e. intercellular adhesion molecule 1, vascular cell adhesion molecule 1, flow mediated dilation, reactive hyperemia index) and markers of inflammation (i.e. interleukins, cytokines).

CONCLUDING REMARKS AND PERSPECTIVES

In the last years, berries have been the object of several studies for their role in human health and prevention of several degenerative diseases. The following review summed the main evidence,
deriving from acute and chronic human berry intervention studies, on their protective effects against oxidative stress. A total of forty-nine studies was analyzed. Studies were performed in healthy subjects but also in those with CVD risk factors, fatty liver diseases, metabolic syndrome, diabetes, hypertension, hyperlipidemia and cancer. Some studies present limitations due to non-randomization and/or lack of control group or control/placebo food, use of non-validated markers, surrogate markers or the use of non appropriate techniques for their evaluation, lack of a complete characterization of food matrix. Moreover, by considering the differences between types of berry and their bioactive composition, the dose, the form (i.e. juice or whole fruit), the application of different methodologies for the evaluation of the biomarkers (direct methods versus indirect methods), made it difficult to compare the results obtained among studies. Greater effort in the application of easy, accurate, robust and shared methods is needed in order to promote more rapid and productive comparisons of research findings across different studies. However, though limited, there are indications that the consumption of berries may protect against protein and lipid oxidation, and increase total plasma and serum antioxidant status in humans. Their effects are observed following chronic interventions both in healthy and unhealthy subjects and in those with cardiovascular risk factors, while for acute studies, results they are inconclusive and inconsistent. Furthermore, results on the effects of berries on DNA damage and endogenous antioxidant enzyme activity are still inconsistent both in acute and chronic intervention. In conclusion, much remains to be elucidated before a comprehensive understanding of the beneficial effect of berries on oxidative stress markers in humans. In this regard, the development of robust and well controlled clinical studies is encouraged.
**Figure 1:** A flow chart highlighting study selection

**Legend**

*Studies were identified according to the following keywords: ‘humans’, ‘berry’, ‘oxidative stress’, ‘DNA damage’, ‘lipid damage’, ‘protein damage’ and ‘antioxidant capacity’*

**Studies were excluded with the following reasons: 1) use of a mix of fruit and vegetable for the intervention in which berries were not the main food; 2) use of soft fruits for the control/placebo intervention*
Table 1: Role of berries in the modulation of oxidative stress biomarkers: overview of the acute human intervention studies

<table>
<thead>
<tr>
<th>References</th>
<th>Study design</th>
<th>Study population</th>
<th>Berry intervention</th>
<th>Control intervention</th>
<th>Outcome measure</th>
<th>Principal finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marniemi et al.</td>
<td>Baseline and post intervention</td>
<td>Six healthy men (mean age, 48.7 years; BMI&lt;30 kg/m²)</td>
<td>240 g of bilberries, lingonberries or blackcurrants (80 g of each)</td>
<td>None</td>
<td>Antioxidant status measured in the LDL fraction by TRAP assay</td>
<td>Increase in antioxidant status</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Composition: Not available</td>
<td></td>
<td>LDL diene conjugation</td>
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<td></td>
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<td></td>
<td>Control supplement</td>
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<td>Serum antioxidant status measured by ORAC assay</td>
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<td></td>
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<td></td>
<td>Control supplement dissolved in 500 mL water with a high-fat meal</td>
<td></td>
<td>Total plasma antioxidant status (TAS)</td>
<td></td>
</tr>
<tr>
<td>Kay and Holub</td>
<td>Single-blind, controlled, crossover</td>
<td>Eight male subjects (mean age, 46.9 ± 1.9 years; mean BMI, 23.8 ± 0.8 kg/m²)</td>
<td>Wild blueberry: 100 g freeze-dried wild blueberry powder dissolved in 500 mL water with a high-fat meal</td>
<td>Control supplement</td>
<td>Increase in serum and plasma antioxidant status</td>
<td></td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td></td>
<td>Composition (100 g): Total phenolics: 2790 mg; ACNs: 1160 mg; Vitamin C: 10 mg; ORAC: 14.7 mmol TE</td>
<td>Control supplement</td>
<td>Serum antioxidant status measured by ORAC assay</td>
<td></td>
</tr>
<tr>
<td>Mazza et al.</td>
<td>Single-blind, controlled, crossover</td>
<td>Five male subjects (mean age, 46.9 ± 1.9 years; mean BMI, 23.8 ± 0.8 kg/m²)</td>
<td>Blueberry: 100g freeze-dried + high fat meal</td>
<td>Control supplement</td>
<td>Serum antioxidant capacity measured by TEAC and ORAC</td>
<td>Increase in serum antioxidant capacity</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td></td>
<td>Composition (100 g): Total phenolics: 2790 g; ACNs: 1160 mg; Vitamin C: 10 mg; ORAC: 14.7 mmol TE</td>
<td>Control supplement</td>
<td>Serum antioxidant capacity measured by TEAC and ORAC</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Intervention Type</td>
<td>Participants</td>
<td>Intervention Description</td>
<td>Control Description</td>
<td>Outcome</td>
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<tr>
<td>Netzel <em>et al.</em> 55</td>
<td>Parallel intervention</td>
<td>Antioxidant-rich juice: six healthy volunteers</td>
<td>Antioxidant-rich juice (400 mL) containing 30% white grape, 25% blackcurrant-, 15% elderberry-, 10% sour cherry-, 10% blackberry- and 10% aronia-juice</td>
<td>Tap water (400 mL)</td>
<td>Increase in plasma antioxidant capacity</td>
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<td>Control group (2 females / 2 males). Subjects characteristic not available.</td>
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<td>Composition (400 mL): Total phenols: 988 mg GAE; ACNs: 166 mg; Ascorbic acid: 41.2 mg; TEAC: 16.4 mmol TE/L</td>
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<tr>
<td>Morillas-Ruiz <em>et al.</em> 56</td>
<td>Randomized, counterbalanced - double-blind intervention</td>
<td>Antioxidant group: 13 healthy cyclists (mean age, 23±5 years; mean BMI, 23.7±3.9 kg/m²)</td>
<td>Antioxidant beverage (3 mL/kg body weight) containing black grape (81.2 g/L), raspberry (93.0 g/L) and red currant (39.2 g/L)</td>
<td>Placebo beverage (3 mL/kg) without antioxidants and berries but identical in appearance and taste.</td>
<td>Increase in 8-OHdG in the placebo group with no effect on MDA plasma levels</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Placebo group: 13 healthy cyclists (mean age, 25±7 years; mean BMI, 22.6±4.2 kg/m²)</td>
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<td>Composition (1L): Total phenolics: 1.41 mg; (ACNs 60%, Hydroxycinnamic acid esters 19%, Ellagic acid 13%, Flavonols: 6%);</td>
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<tr>
<td>Study</td>
<td>Intervention Type</td>
<td>Subjects</td>
<td>Intervention Details</td>
<td>Measurement Details</td>
<td>Increase in Plasma Antioxidant Capacity</td>
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</tr>
<tr>
<td>Netzel et al.</td>
<td>Baseline and post-prandial intervention (each subject acted as own control)</td>
<td>Eight healthy, non-smoking subjects (4 females/4 males; range age, 24-34 years; mean BMI, 22.6 ±2.8 kg/m²)</td>
<td>Elderberry juice: (400 mL) (400 mL): Total phenolics: 2240 mg GAE; Total ACNs: 710 mg; Ascorbic acid: 16 mg; Antioxidant capacity: 9.16 mmol (TEAC assay) and 16.8 mmol (TRAP assay)</td>
<td>Control: 400 mL water</td>
<td>Increase in total plasma antioxidant capacity</td>
<td></td>
</tr>
<tr>
<td>Vinson et al.</td>
<td>Baseline and post-prandial intervention</td>
<td>Ten healthy subjects (7 females/3 males; range age, 25-38 years; range BMI, 22-33 kg/m²)</td>
<td>Cranberry juice: (240 mL; 27% cranberry juice + high fructose corn syrup containing 21 g of glucose and 17 g of fructose + 80 mg of vitamin C) (240 mL): Catechins: 175 mg CE; Cyanidin: 16 mg; Peonidin: 8 mg; Quercetin: 3 mg</td>
<td>Control juice: same characteristics of high fructose corn syrup + 80 mg of vitamin C</td>
<td>Total plasma antioxidant capacity measured by FRAP assay</td>
<td>Increase in total plasma antioxidant capacity</td>
</tr>
<tr>
<td>Tulipani et al.</td>
<td>Baseline and post-prandial intervention</td>
<td>Eight healthy subjects (5 females/3 males; mean age, 30±6 years; mean BMI, 24±2 kg/m²)</td>
<td>Six different strawberry cultivars (1kg) (kg FW, range among 6 cultivars): Vitamin C: 0.28-0.40 g; Folate: 128-</td>
<td>None</td>
<td>Total plasma antioxidant capacity measured by TEAC and FRAP assay</td>
<td>Increase in total plasma antioxidant capacity</td>
</tr>
<tr>
<td>Burton-Freeman et al., 61</td>
<td>Randomized, single-blind, placebo-controlled, crossover intervention. Baseline and post-prandial intervention</td>
<td>Twenty-four hyperlipidemic subjects (14 females/10 males; mean age, 50.9 ± 15 years; mean BMI, 29.2 ± 2.3 kg/m²)</td>
<td>High-fat test meal + strawberry beverage (containing a mixture of cultivated strawberry in freeze-dried form). The beverages contained 10 g/serving, which was equivalent to 110 g/die of fresh strawberries. Composition (10g): Total phenolics: about 338 mg</td>
<td>High-fat test meal + Placebo strawberry-flavored beverage prepared from non-strawberry ingredients to provide, as close as possible, the total energy (calories), macro- and micronutrient content, and fiber content, without bioactive compounds</td>
<td>ox-LDL plasma levels</td>
<td>Reduction of ox-LDL plasma levels</td>
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<tr>
<td>Rosenblat et al., 62</td>
<td>Cross-over intervention</td>
<td>Six healthy male subjects (range age, 25–30 years; BMI, not available)</td>
<td>Polyphenolic-rich beverages (250 mL/day): 1-acai juice blend (Total polyphenols: 1100 mg GAE); 2 – 100% Concord grape juice (Total</td>
<td>None (baseline values acted as control)</td>
<td>Total thiols (SH) protein group in serum ox-LDL plasma levels and serum lipid peroxidation</td>
<td>No effect on SH protein group levels Reduction in plasma ox-LDL and serum lipid peroxidation following black currant juice</td>
</tr>
<tr>
<td>Study</td>
<td>Intervention</td>
<td>Participants</td>
<td>Beverage Composition</td>
<td>Plasma Antioxidant Capacity</td>
<td>DNA Damage</td>
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<tr>
<td>McLeay et al., 64</td>
<td>Randomized, placebo-controlled, crossover intervention</td>
<td>Ten healthy physically active females (mean age, 22 ± 1 years; BMI, not available)</td>
<td>Blueberry beverage: 200 g frozen blueberries, 50 g banana, and 200 mL commercial apple juice  Composition (100 mL):  Total phenolics: 168 mg GAE; ACNs: 96.6 mg; Phenolic acids: 26 mg; Flavonoids: 10.2 mg; Vitamin C: 45 mg; Vitamin E: 3 mg; ORAC: 5.4 mmol TE</td>
<td>Total plasma antioxidant capacity measured by FRAP assay  Plasma protein carbonyl plasma levels</td>
<td>Increase in plasma antioxidant capacity  No effect on protein carbonyl plasma levels</td>
<td></td>
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<tr>
<td>Del Bo et al., 65</td>
<td>Randomized, controlled, crossover intervention</td>
<td>Ten young healthy subjects (mean age, 20.8 ± 1.6 years; mean BMI, 22.5 ± 2.1 kg/m²)</td>
<td>Blueberry purée (300 g)  Composition (300 g):  Total phenolics: 727.2 mg GAE; ACNs: 348.3 mg</td>
<td>DNA SBs (without FPG or H₂O₂), FPG-sensitive sites and H₂O₂-induced DNA damage in PBMCs.</td>
<td>Reduction in H₂O₂-induced DNA damage  No effect on DNA SBs and FPG-sensitive sites</td>
<td></td>
</tr>
</tbody>
</table>
| Mathison et al., 66 | Randomized, double-blind, placebo-controlled crossover intervention | Twelve subjects (6 females/6 males; mean age, 27.5 ± 1.3 years; mean BMI, 23.7 ± 0.9 kg/m²) | Cranberry leaf extract beverage: 15.2 oz (about 456 mL)
Composition (15.2 oz): Vitamin C: nd; Proanthocyanidins: 119 mg; Total phenolics: 111 mg; Total ACNs: nd
Low-calorie cranberry juice cocktail: 16 oz (about 480 mL)
Composition (16 oz): Vitamin C: 100 mg; Proanthocyanidins: 192 mg; Total phenolics: 338 mg; Total ACNs: 17.4 mg cyanidin-3-galactoside) | Placebo: 15.2 oz (about 456 mL)
Composition: Vitamin C: nd; Proanthocyanidins: nd; Total phenolics: 19 mg; Total ACNs: nd | Plasma total antioxidant power
Plasma levels of 8-OHdG
SOD and GSH-Px activity in red blood cells | No effect of intervention on total plasma antioxidant power and 8-OHdG plasma levels
Increase in GSH-Px activity following cranberry leaf extract consumption
Increase in SOD activity following low-calorie cranberry juice consumption |
Table 2: Role of berries in the modulation of oxidative stress biomarkers: overview of the chronic human intervention studies

<table>
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<tr>
<th>References</th>
<th>Duration of intervention</th>
<th>Study design</th>
<th>Study population</th>
<th>Berry intervention</th>
<th>Control intervention</th>
<th>Outcome measures</th>
<th>Principal findings</th>
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</thead>
<tbody>
<tr>
<td>Marniemi et al., 52</td>
<td>8 weeks</td>
<td>Parallel intervention</td>
<td>Sixty men, 60 years old, BMI&lt;30 kg/m²</td>
<td>Berry group: 100 g/day of bilberries, lingonberries or blackcurrants in any order</td>
<td>Control group: 500 mg calcium gluconate</td>
<td>Antioxidant capacity measured in LDL fraction by TRAP assay</td>
<td>No effect on LDL antioxidant capacity following berries</td>
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<td>Supplement group: supplement of 100 mg/day of Tocopherol, and 500 mg/day of Ascorbic acid</td>
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<td>Total and LDL serum diene conjugation</td>
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<td></td>
<td>Control group: 500 mg calcium gluconate</td>
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<tr>
<td>Murkovic et al., 69</td>
<td>3 weeks</td>
<td>Randomized, placebo- controlled parallel intervention</td>
<td>Fourteen healthy subjects (7 females/7 males)</td>
<td>Elderberry group: capsules (400mg spray-dried powder containing 10% ACNs; equivalent to 5 mL elderberry juice)</td>
<td>Placebo group: capsules (400mg spray-dried powder ACNs free)</td>
<td>Ox-LDL plasma levels</td>
<td>No effect on ox-LDL plasma levels</td>
</tr>
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<td></td>
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<td>Elderberry group: capsules (400mg spray-dried powder containing 10% ACNs; equivalent to 5 mL elderberry juice)</td>
<td>Composition: ACNs: 100 mg</td>
<td>Composition: No ACNs</td>
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<td>Placebo group: 7 subjects (subjects characteristics not available)</td>
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<td></td>
<td></td>
<td>Placebo group: 7 subjects (subjects characteristics not available)</td>
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<tr>
<td>Møller et al., 70</td>
<td>3 weeks</td>
<td>Randomized, controlled parallel intervention</td>
<td>Eighteen (11 females / 7 males) healthy subjects for blackcurrant juice (range age, 20-45 yeas; range BMI, 19.6–29.1)</td>
<td>Blackcurrant drink (475 to 1000 mL/day according to body weight) ACNs: 397 mg/day; Vitamin C: 140</td>
<td>Control drink (475 to 1000 mL/day according to body weight) No ACNs and vitamin C</td>
<td>SB, Endo III and FPG- sensitive sites in MNBCs</td>
<td>Increase in FPG-sensitive sites. No effect on DNA SB and Endo III sensitive sites in MNBCs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control drink (475 to 1000 mL/day according to body weight) No ACNs and vitamin C</td>
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</tbody>
</table>

SB, Endo III and FPG-sensitive sites in MNBCs.
<table>
<thead>
<tr>
<th>Study</th>
<th>Duration</th>
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<th>Participants</th>
<th>Intervention</th>
<th>Outcome Measures</th>
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</thead>
<tbody>
<tr>
<td>Ruel et al., 72</td>
<td>2 weeks</td>
<td>Baseline and post-intervention</td>
<td>Twenty (14 females / 6 males) healthy subjects for ACN drink (range age, 21-52 years; range BMI, 19.1-27.2 kg/m²)</td>
<td>ACN drink: 475 to 1000 mL/day according to body weight) ACNs: 365 mg/day</td>
<td>Increase in total plasma antioxidant capacity</td>
</tr>
<tr>
<td>McAnulty et al., 73</td>
<td>3 weeks</td>
<td>Randomized, controlled, parallel intervention</td>
<td>Nineteen (12 females / 7 males) healthy subjects for control intervention (range age, 19-46 years; range BMI, 19.1-28.1 kg/m²)</td>
<td>None</td>
<td>Reduction in ox-LDL plasma levels</td>
</tr>
<tr>
<td>Wilms et al., 74</td>
<td>4 weeks</td>
<td>Baseline and post-intervention</td>
<td>Eight healthy non-smokers</td>
<td>Blueberry juice and apple juice (50:50)</td>
<td>Increase in total plasma antioxidant capacity</td>
</tr>
<tr>
<td>Study</td>
<td>Duration</td>
<td>Intervention Details</td>
<td>Participants</td>
<td>Intervention Details</td>
<td>Outcome Measures</td>
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<tr>
<td>Duthie et al., 76</td>
<td>2 weeks</td>
<td>Randomized, controlled, parallel intervention</td>
<td>Twenty healthy females (range age, 18–40 years) Cranberry group (11 subjects; mean age, 27.3±6.5 years; BMI, not available) Placebo group (9 subjects; mean age, 28.3 ± 7.5 years; BMI, not available)</td>
<td>Cranberry juice (750 mL/day) Composition (750 mL): Vitamin C: 672.75 mg; Total phenols: 852 mg GAE; Catechins: 21.8 mg; ACNs: 2.1 mg as malvidin-3-glycoside equivalents; Antioxidant capacity: 10.5 mmol FeII (FRAP assay) Placebo drink (750 mL/day) prepared with natural mineral water, strawberry flavor, sucrose (9 g/100 mL) Composition (750 mL): Placebo drink: Vitamin C (1.38 mg), Total phenols (6.72 mg GAE) ACNs: nd; TAC: 0.045 mmol FeII (FRAP assay)</td>
<td>Total plasma antioxidant capacity measured by FRAP and ESR assay (Fremy’s radical reduction) GSH-Px, CAT, and SOD activity in erythrocytes DNA SB, H₂O₂-induced DNA damage, Endo III sensitive bases in lymphocytes, 8-OHdG in urine. No effect on the levels of DNA damage</td>
</tr>
<tr>
<td>Kresty et al., 76</td>
<td>6 months</td>
<td>Baseline and post-intervention</td>
<td>Twenty males and females (range age, 48–68 years, range BMI, 24.84–40.97 kg/m²) with Barrett’s esophagus (premalignant Esophageal condition)</td>
<td>Black raspberries drink (170 mL/day) prepared suspending 32 g for females and 45 g for males of lyophilized black raspberries in 170 mL of water Composition (100 g): Total phenolics: 5938 mg; Ellagic acid: 185 mg; None</td>
<td>Urine levels of 8-OHdG No effect on urine levels of 8-OHdG (only 10 subjects completed the study)</td>
</tr>
</tbody>
</table>
| Valentová et al.,18 | 8 weeks | Randomized, placebo-controlled, parallel intervention | Dried cranberry group I: 20 healthy young women (mean age 21.4± 2.0 years; mean BMI, 21.2 ±1.5 kg/m²)
Dried cranberry group II: 22 healthy young women (mean age, 21.7±2.0 years; mean BMI, 20.5±1.8 kg/m²)
Placebo group: 23 healthy young women (mean age, | Dried cranberry group I: 400 mg (two capsules once)/day
Composition (400 mg):
Total polyphenolic content: 12 mg; Quercetin: 1.2 mg; ACNs: 1.76 mg
Dried cranberry group II: 1200 mg (two capsules three times)/day
Composition (1200 mg):
Total polyphenolic | Placebo group: (two capsules/day) | Serum levels of oxidation protein Products (AOPP), SH group
MDA serum and erythrocyte levels
SOD and GSH-Px erythrocyte activity | Reduction in AOPP and SH group levels following cranberry (1200 mg/die) intervention.
Reduction in MDA serum levels but increase in erythrocytes following cranberry (1200 mg/die) intervention.
No effect on SOD and GSH-Px activity.

Vitamin C: 2 mg;
ORAC: 60.1 mmol TE
For men:
Total phenolics: 2672.1 mg; Ellagic acid: 83.25 mg;
Vitamin C: 0.9 mg;
ORAC: 60.1 mmol TE
For women:
Total phenolics: 1900.16 mg; Ellagic acid: 59.2 mg;
Vitamin C: 0.64 mg;
ORAC: 60.1 mmol TE
<table>
<thead>
<tr>
<th>Study</th>
<th>Duration</th>
<th>Group Details</th>
<th>Intervention Details</th>
<th>Outcome Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilms et al.79</td>
<td>4 weeks</td>
<td>One hundred sixty-eight healthy nonsmokers subjects (114 females/54 males; range age, 18-45 years; BMI, not available)</td>
<td>Blueberry/apple juice 50:50 (1 L/day) Composition (1L): Quercetin: 97 mg; Ascorbic acid: 16 mg</td>
<td>None Total plasma antioxidant capacity measured by TEAC assay H₂O₂-induced DNA damage in PBMCs Increase in plasma total antioxidant status Increase protection against <em>ex vivo</em> H₂O₂-oxidative DNA damage</td>
</tr>
<tr>
<td>Jenkins et al.80</td>
<td>30 days</td>
<td>Twenty-eight hyperlipidemic subjects (range age, 38-75 years; range BMI, 19.8-32.3 kg/m²)</td>
<td>Strawberries (454 g/day) Composition: Not available</td>
<td>Oat bran bread (65 g/day) as control diet Serum levels of reduced thiol (–SH) groups as a measure of protein oxidation Plasma levels of MDA equivalent (TBARS) and conjugated dienes Reduction in oxidative protein damage following both interventions Reduction in MDA plasma levels No effect on plasma levels of conjugated dienes</td>
</tr>
<tr>
<td>Lee et al.81</td>
<td>12 weeks</td>
<td>Cranberry group: Fifteen subjects with type 2 diabetes (6 females/9 males; mean age, 65±2 years; mean BMI, 26.2±0.7 kg/m²) Control group: Fifteen subjects with type 2 diabetes (8 females /7 males; mean age, 66±2 years; mean BMI,</td>
<td>Cranberry extracts (one capsule 500mg/day) Composition: Not available</td>
<td>Placebo capsules (500mg/day) ox-LDL plasma levels No effect on ox-LDL plasma levels</td>
</tr>
<tr>
<td>Study</td>
<td>Duration</td>
<td>Design</td>
<td>Participants</td>
<td>Intervention</td>
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<tr>
<td>Ruel et al., 82</td>
<td>12 weeks</td>
<td>Parallel intervention</td>
<td>Thirty-one men (mean age, 51 ±10 years; mean BMI, 27.8 ± 3.2 kg/m²) with (n=9) and without (n=21) metabolic syndrome</td>
<td>Low-calorie cranberry juice concentrate: 125 mL/day (first 4 weeks), 250 ml/die (second 4 weeks) and 500 ml/die (third 4 weeks)</td>
</tr>
<tr>
<td>Basu et al., 85</td>
<td>4 weeks</td>
<td>Baseline and post-intervention</td>
<td>Sixteen female (range age, 39-71 years; mean BMI, 38.6 ± 2.3 kg/m²) with metabolic syndrome</td>
<td>Two cups of strawberry drink per day (each cup containing 25 g of freeze-dried strawberry powder, one cup of water, one teaspoon of artificial sweetener, and one teaspoon vanilla essence).</td>
</tr>
<tr>
<td>Basu et al., 86</td>
<td>8 weeks</td>
<td>Single-blinded</td>
<td>Forty-eight</td>
<td>Two cups of water</td>
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<thead>
<tr>
<th>Study</th>
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<th>Measured Outcomes</th>
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<tbody>
<tr>
<td>Burton-Freeman et al., 61</td>
<td>6 weeks</td>
<td>Randomized, single-blind, placebo-controlled, crossover intervention</td>
<td>Twenty-four hyperlipidemic subjects (14 females/10 males; mean age, 50.9 ± 15 years; mean BMI, 29.2 ± 2.3 kg/m²)</td>
<td>Strawberry drink containing a mixture of cultivated strawberry in freeze-dried form. The beverages contained 10 g/serving (equivalent to 110 g/day of fresh strawberries). At the study day, (after 6 weeks intervention) subjects consumed a high fat meal</td>
<td>Composition (10 g): Total phenolics: about 338 mg</td>
</tr>
<tr>
<td>Henning et al., 83</td>
<td>3 weeks</td>
<td>Baseline and crossover</td>
<td>Twenty-one</td>
<td>None</td>
<td>Serum GST activity</td>
</tr>
<tr>
<td>Study</td>
<td>Duration</td>
<td>Intervention Type</td>
<td>Subjects</td>
<td>Intervention Details</td>
<td>Measured Parameters</td>
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<tr>
<td>Karlsen et al., 83</td>
<td>4 weeks</td>
<td>Randomized, controlled, parallel intervention</td>
<td>Berry group: Thirty-one subjects (10 females / 21 males; range age, 34–68 years; range BMI, 19.9-31.7 kg/m²) with elevated levels of at least one risk factor for CVD  Control group: Thirty-one subjects (7 females/25 males; range age, 30–68 years; range BMI, 17.8-31.5 kg/m²) with elevated levels of at least one risk factor for CVD</td>
<td>Bilberry juice: 330 mL/day diluted to 1 L using tap water  Control group: Water (1 L/day)</td>
<td>DNA SBs, 8-OHdG and dG in DNA of mononuclear lymphocytes  ox-LDL plasma levels</td>
</tr>
<tr>
<td>Rosenblat et al., 62</td>
<td>1 week</td>
<td>Cross-over intervention</td>
<td>Six healthy male subjects (range age, 25–30 years; BMI, not available)</td>
<td>Polyphenolic-rich beverages (250 mL/day): 1-acai juice blend (Total)</td>
<td>Total plasma antioxidant capacity measured by FRAP, TRAP and ORAC assay  Plasma lipid peroxidation measured by Diacrons reactive oxygen metabolites</td>
</tr>
<tr>
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<td>Design</td>
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<td>Intervention Details</td>
<td>Primary Outcome Measures</td>
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<tr>
<td>Basu et al., 89</td>
<td>8 weeks</td>
<td>Randomized, double-blind, placebo-controlled, parallel intervention</td>
<td>Thirty-one females (mean age, 52.0 ± 8.0 years; mean BMI, 40.0 ± 7.7 kg/m²) with metabolic syndrome</td>
<td>Low energy cranberry juice (480 mL/day) Composition (480 mL): Vitamin C: 120 mg; Total phenolics: 458 mg; Proanthocyanidins: 119.0 mg; ACNs: 24.8 mg Placebo (480 mL/day) was identical to cranberry juice but free of phenolic compounds</td>
<td>Plasma antioxidant capacity measured by metmyoglobin assay Plasma levels of oxidized LDL; serum levels of MDA and HNE</td>
</tr>
<tr>
<td>Lee et al., 90</td>
<td>4 weeks</td>
<td>Baseline and post-intervention</td>
<td>Fifteen healthy subjects (mean age, 24.3 ± 1.9 years; mean BMI, 22.1 ± 1.7 kg/m²)</td>
<td>Supplements containing 30 g of freeze-dried raspberry Composition (30 g): Vitamin E: 2.88 mg; Phenols: 315 mg; Vitamin A: 0.39 mg None</td>
<td>Plasma levels of MDA equivalent (TBARS)</td>
</tr>
<tr>
<td>McAnulty et al., 91</td>
<td>6 weeks +</td>
<td>Randomized, Blueberry group:</td>
<td>Fresh blueberry No blueberry</td>
<td>Total plasma</td>
<td>Increase in total</td>
</tr>
<tr>
<td>Study</td>
<td>Duration</td>
<td>Design</td>
<td>Participants</td>
<td>Intervention</td>
<td>Outcome Measures</td>
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<tr>
<td>Tulipani et al., 92</td>
<td>16 days</td>
<td>Baseline and post-intervention</td>
<td>Twelve healthy individuals (7 females/ 5 males; mean age, 34 ± 8 years; mean BMI, 22.2 ± 2.4 kg/m²)</td>
<td>Fresh strawberry (500g/day) Composition (500 g): Vitamin C: 0.18 g; Total folate: 119.4 μg; Total phenols: 1.9 g GAE; Total flavonoids: 0.4 g; Total ACNs: 150 mg; Antioxidant capacity: 6.1 mmol TE (FRAP assay) and 20.3 mmol TE (TEAC assay)</td>
<td>None Total plasma antioxidant capacity measured by TEAC and FRAP assay Plasma levels of conjugated dienes Increase in plasma antioxidant capacity, measured by FRAP but not TEAC assay No effect on plasma levels of conjugated dienes</td>
</tr>
<tr>
<td>Moazen et al., 93</td>
<td>6 weeks</td>
<td>Randomized double-blind</td>
<td>Thirty-six subjects (23</td>
<td>Freeze-dried strawberry (50g), Placebo powder (40g/day),</td>
<td>Total serum antioxidant status Increase in total serum antioxidant status</td>
</tr>
</tbody>
</table>
controlled parallel intervention

females / 13 males) with type 2 diabetes (mean age, 51.57 ± 10 years; mean BMI, 27.90 ± 3.7 kg/m²)

Freeze-dried strawberry group:
19 diabetic subjects (mean age, 51.88 ± 8.26 years; mean BMI, 27.32 ± 3.26 kg/m²)

Placebo group:
17 diabetic subjects (mean age, 51.17 ± 13.88 years; mean BMI, 28.70 ± 4.24 kg/m²)

identical to 500g fresh strawberries/day

equivalent to 500g fresh strawberries/day

Composition (50 g):
Total phenolics: 2006 mg GAE; total ACNs: 154mg CGE; Vitamin C: 109mg

identical to strawberry powder in appearance, containing 4g of pectin, 12g of lactose, 4g of instant sugar-free drink powder with strawberry flavor

measured by ORAC assay

Serum levels of MDA equivalent (TBARS)

Reduction in MDA serum levels

Riso et al., 94

6 weeks

Randomized, controlled, crossover intervention

Twenty subjects with cardiovascular risk factors (mean age, 47.8 ± 9.7 years; mean BMI, 24.8 ± 2.6 kg/m²)

Wild blueberry drink (250 mL/day natural mineral water, 25g wild blueberry powder)

Composition (250 mL):
ACNs: 375 mg; Vitamin C: 4.2 mg

Placebo drink:
natural mineral water (250 mL/day) with blueberry flavor, colorants, sugars

GST, SOD and GSH-Px activity in red blood cells

DNA SBs (without FPG or H₂O₂), FPG-sensitive sites and H₂O₂-induced DNA damage, DNA repair capacity in PBMCs

No significant effect on GST, SOD and GSH-Px activity

Reduction in FPG-sensitive sites and H₂O₂-induced DNA damage. No effect on DNA SBs and DNA repair capacity

Ruel et al., 95

4 weeks

Randomized, placebo-controlled

Thirty-five men (mean age, 45 ± 10 years; mean

Low-calorie cranberry juice concentrate (27%)

Placebo juice (500 mL/day) of water + vitamin C. Taste,
ox-LDL plasma levels

No effect on ox-LDL plasma levels
<table>
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<tr>
<th>Study</th>
<th>Duration</th>
<th>Baseline and post-intervention</th>
<th>Intervention Details</th>
<th>Composition</th>
<th>Plasma levels</th>
<th>Reduction in oxidative stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simão et al.</td>
<td>60 days</td>
<td>Cranberry group: 20 subjects with metabolic syndrome (14 females/6 males; range age, 42.0–53.0 years; range BMI, 26.3–38.4 kg/m²) Control group: 36 subjects with metabolic syndrome (28 females/8 males; range age, 44.8–56.3 years; range BMI, 31.32–36.9 kg/m²)</td>
<td>Cranberry juice (700 mL/day)</td>
<td>Vitamin C: 210 mg; Proanthocyanidins: 231 mg; Total phenolics: 364 mg; Folic acid: 0.42 mg; TEAC: 128.6 mmol TE</td>
<td>None</td>
<td>Plasma levels of AOPP, Plasma levels of lipid hydroperoxides, Reduction in lipoperoxidation</td>
</tr>
<tr>
<td>Alvarez-Suarez et al.</td>
<td>30 days</td>
<td>Twenty-three healthy volunteers (12 females/11 males; mean age, 27 ± 3.2 years; mean BMI, 21.74 ± 2.5 kg/m²)</td>
<td>Fresh strawberry: (500g/day)</td>
<td>None</td>
<td>Total plasma antioxidant capacity measured by FRAP and ORAC assay, Reduction in 8-OHdG urine levels</td>
<td>Increase in plasma total antioxidant capacity, Reduction in 8-OHdG urine levels</td>
</tr>
</tbody>
</table>
| Basu et al., 28 | 3 months | Randomized, controlled, parallel intervention | Sixty volunteers with CVD risk factors  
LD-FDS: 15 subjects (14 females / 1 male; mean age, 50 ± 10 years; mean BMI, 34.5 ± 4.4 kg/m²)  
HD-FDS: 15 subjects (13 females / 2 males; mean age, 49 ± 11 years; mean BMI, 38.0 ± 7.1 kg/m²)  
LD-C: 15 subjects (14 females / 1 male; mean age, 48 ± 10 years; mean BMI, 37.0 ± 4.4 kg/m²)  
HD-C: 15 subjects (14 females / 2 male; mean age, 48 ± 10 years; mean BMI, 35.0 ± 5.2 kg/m²) | Flavonoids: 470 mg; Antioxidant capacity: 5.53 μmol TE/L (FRAP assay) and 24.8 μmol TE/L (ORAC assay) | Plasma MDA and urinary isoprostanes levels | Reduction in MDA plasma levels and urinary isoprostanes |
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<td>Low dose freeze-dried strawberries (LD-FDS): 25 g of freeze-dried powder reconstituted in 2 cups (474 mL/day) of water daily (corresponding to 250 g of fresh strawberries); Composition (25 g): Vitamin C: 55 mg; Total phenolics: 1001 mg GAE; ACNs: 78 mg; Ellagic acid: 106 mg</td>
<td>Low-dose calorie- and fiber-matched control (LD-C): 4 g of fiber and 5 teaspoons (20 g) of cane sugar, blended in 2 cups (474 mL/day) of water. Red food color and artificial strawberry-flavored was added to mimic the color and flavor of the FDS beverages</td>
<td>MDA serum levels</td>
</tr>
</tbody>
</table>
|                |          |                                          | High dose freeze-dried strawberries (HD-FDS): 50 g of freeze-dried powder reconstitute in 2 cups (474 mL/die) of water (corresponding to 500 g of fresh strawberries)  
Composition (50 g): Vitamin C: 109 mg; Total phenolics: 2010 mg GAE | High-dose calorie- and fiber-matched control (HD-C): 8 g of fiber and 9 teaspoons (36 g) of cane sugar, blended in 2 cups (474 mL/day) of water. Red food color and artificial strawberry-flavored was added to mimic the color and flavor of the FDS beverages | MDA serum levels | Reduction in MDA serum levels |
<table>
<thead>
<tr>
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<th>Intervention</th>
<th>Outcome Measures</th>
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<tr>
<td>Kardum et al., 99</td>
<td>3 months</td>
<td>Baseline and post-intervention</td>
<td>Twenty-five healthy women subjects (mean age, 35.2 ± 7.7 years; BMI, not available)</td>
<td>Polyphenol-rich chokeberry juice (100 mL/day)</td>
<td>None</td>
<td>SOD and GSH-Px activities in red blood cells</td>
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<td>Composition (100 g): Total phenolics: 386 mg GAE; Total proanthocyanidins: 442 mg CE; Total ACNs: 153.9 mg; Chlorogenic acids: 60 mg; Total quercetin glycosides: 18 mg</td>
<td></td>
<td>Plasma levels of MDA equivalent (TBARS)</td>
</tr>
<tr>
<td>Kaume et al., 100</td>
<td>9 months</td>
<td>Randomized, controlled, parallel intervention</td>
<td>Forty-five postmenopausal smokers and twenty postmenopausal nonsmokers NS group (non-smokers group): 20 postmenopausal (mean age, 58.0 ± 0.9 years; mean BMI, 23.7 ± 1.5 kg/m²) SC group (control group): 21 postmenopausal smokers (mean age, 54.4 ± 0.9</td>
<td>S-BB group: 45 g/day freeze-dried blackberry</td>
<td>Control group was only directed to maintain their usual diet</td>
<td>Serum levels of MDA equivalent (TBARS)</td>
</tr>
</tbody>
</table>
### Study Details

**Khan et al., 101**

- **Duration**: 6 weeks
- **Design**: Randomized, double-blind, placebo-controlled, parallel intervention
- **Intervention Groups**:
  - Low blackcurrant group: Twenty-two healthy subjects (7 females / 15 males; mean age, 55 ± 10 years; mean BMI, 28.4 ± 5.4 kg/m²)
  - High blackcurrant group: Twenty-one healthy subjects (8 females / 13 males; mean age, 51 ± 11 years; mean BMI, 29.2 ± 6.9 kg/m²)
- **Placebo Group**: Twenty-two healthy subjects (7 females / 15 males; mean age, 54.3 ± 1.6 years; mean BMI, 25.2 ± 2.5 kg/m²)

### Intervention Details

- **Low (6.4% juice) blackcurrant drink (250 mL/4 times per day)**
  - Composition (250 mL):
    - Vitamin C: 2.75 mg
    - Total phenolics: 68.25 mg
    - ACNs: 10 mg

- **High (20% juice) blackcurrant drink (250 mL/4 times per day)**
  - Composition (250 mL):

- **Placebo drink (flavored water, 250 mL/4 times per day)**

### Outcomes

- **Isoprostanes plasma levels**
  - Reduction in isoprostanes plasma levels only following intervention with high blackcurrant drink
<table>
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<tr>
<th>Placebo group: Twenty-one healthy subjects (6 females/15 males; mean age, 51 ± 8 years; mean BMI, 28.9 ± 6.5 kg/m²)</th>
<th>mL: Vitamin C: 25.5 mg; Total phenolics: 203.75 mg; ACNs: 35.75 mg</th>
</tr>
</thead>
</table>

**Kuntz et al.**<sup>102</sup> 2 weeks Randomized, double-blind, placebo-controlled, crossover intervention Thirty healthy female subjects (range age, 23-27 years; range BMI, 18.2-27.9 kg/m²) ACN-rich juice: 80% red grape and 20% bilberry juice (0.33 L/day) Composition (0.33 L): Ascorbic acid: 33.66 mg; Total phenolics: 1064.91 mg CE; ACNs: 277.2 mg ACN-rich smoothie: 80% red grape purée and 20% bilberry purée (0.33 L/die) Composition (0.33L): Ascorbic acid: 38.94 mg; Total phenolics: 1133.55 mg CE; ACNs: 324.39 mg Placebo juice: 100% grape juice 0.33 L/day Composition (0.33 L): Ascorbic acid: 7.26 mg; Total phenolics: 59.07 mg CE; ACNs: 2.96 mg Plasma antioxidant capacity measured by TEAC assay Plasma SOD, GSH-Px, CAT and erythrocyte SOD activities 8-OH-dG urine levels Plasma and urine levels of MDA equivalent (TBARS) Increase in plasma antioxidant capacity Increase in plasma SOD and CAT activity No effect on plasma GSH-Px and erythrocyte SOD activity No effect on 8-OH-dG urine levels Reduction in plasma and urinary MDA levels

**Guo et al.**<sup>103</sup> 4 weeks Randomized, placebo-controlled, double-blind, crossover intervention Forty-four subjects (males and females; range age, 18–25 years; BMI ≥ 23.1 kg/m²), with fatty Bayberry juice (500 mL/day) Composition (500 mL): Total polyphenols: 1351 mg GAE; Placebo juice (500 mL/day) Composition (500 mL): taste and color similar to bayberry juice. Protein carbonyl groups plasma levels Reduction in protein plasma carbonyl groups following bayberry juice
<table>
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<tr>
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<th>Intervention</th>
<th>Outcome Measures</th>
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<tr>
<td>Soltani <em>et al.</em>, 104</td>
<td>4 weeks</td>
<td>Randomized, double-blind, placebo-controlled, parallel intervention</td>
<td>Whortleberry group: twenty-five hyperlipidemic subjects (15 females / 10 males; mean age, 48.08±16.39 years, mean BMI, 25.40±1.75 kg/m²) Placebo group: twenty-five hyperlipidemic subjects (15 females / 10 males; mean age, 46.36±16.59 years, mean BMI, 25.21±2.01 kg/m²)</td>
<td>Whortleberry capsules (2 capsules/day). Composition (2 capsules): ACNs: 45 mg</td>
<td>MDA serum levels Reduction in MDA plasma levels</td>
</tr>
<tr>
<td>Tulipani <em>et al.</em>, 105</td>
<td>2 weeks</td>
<td>Baseline and post-intervention</td>
<td>Eighteen healthy subjects (10 females / 8 males; mean age, 35 ± 10 years; mean BMI, 23 ± 3 kg/m²)</td>
<td>Fresh strawberry (500g/day) Composition (500 g): Vitamin C: 220 mg; Total folate: 144.6 μg; Total phenols: 700 mg; Total flavonoids: 100 mg; Total ACNs: 318.81 mg; Antioxidant capacity: 6.6 mmol</td>
<td>Total plasma antioxidant capacity measured by TEAC and FRAP assay H₂O₂-induced DNA damage in PBMCs No effect on total plasma antioxidant capacity Reduction in DNA damage measured as comet area, comet length and tail length, while increase in the percentage of DNA in tail</td>
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<tr>
<td>Study</td>
<td>Duration</td>
<td>Design</td>
<td>Participants</td>
<td>Intervention Details</td>
<td>Outcomes</td>
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<tr>
<td>Ivanova et al., 106</td>
<td>30 days</td>
<td>Baseline and post-intervention</td>
<td>Twenty-one healthy subjects (15 female/6 males; mean age, 25.2 ± 10.7 years; mean BMI, 23.1 ± 6.0 kg/m²)</td>
<td>Elderberry drink (200mL/day)</td>
<td>Increase in total serum antioxidant capacity</td>
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<td>Composition (200mL): Total polyphenols: 45.32 mg QE; ACNs: 3.66 mg CGE; Antioxidant capacity: 1.45 mmol (TAC assay)</td>
<td>Serum levels of total thiols group</td>
</tr>
<tr>
<td>Johnson et al., 107</td>
<td>4-8 weeks</td>
<td>Randomized, double-blind, placebo-controlled parallel intervention</td>
<td>Forty-eight postmenopausal women with pre- and stage 1 hypertension Blueberry group: 25 females (mean age, 59.7 ± 4.58 years; mean BMI, 30.1 ± 5.94 kg/m²) Control group: 23 females (mean age, 57.3 ± 4.76 years; mean BMI, 32.7 ± 6.79 kg/m²)</td>
<td>Blueberry drink: freeze-dried blueberry powder (22 g) in 240 mL of water Placebo drink: macronutrient-matched control powder (22 g) in 240 mL of water Composition: no bioactive compounds</td>
<td>Increase in total serum antioxidant capacity</td>
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<td>Serum levels of total thiols group</td>
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<tr>
<td>Bloedon et al., 108</td>
<td>8 weeks</td>
<td>Baseline and post-intervention</td>
<td>Ten untrained males (age range, 20-29 years; mean BMI, 27.0 ± 0.31 kg/m²)</td>
<td>Fresh-frozen steam blanched and puréed wild blueberry (300g)</td>
<td>No effect on manganese-SOD plasma concentration</td>
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<td>Single DNA SBs in plasma No effect on DNA</td>
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<tr>
<td>Composition:</td>
<td>PBMCs</td>
<td>damage in PBMCs</td>
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<td>Not available</td>
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Legend: 5-OHMU: 5-hydroxymethyl-2'-deoxyuridine; 8-OHdG: 8-hydroxy-2’-deoxyguanosine; ACNs: anthocyanins; AOPP: plasma levels of oxidation protein products; BMI: body mass index; CAT: catalase; CE: catechin equivalents; CGE: cyanidine 3-glucoside equivalents; dG: deoxyguanosine; Endo III: endonuclease III; FPG: formamidopyrimidine DNA glycosilase; FRAP: Ferric reducing antioxidant power; FW: fresh weight; GAE: gallic acid equivalents; GSH-Px: glutathione peroxidase; GST: glutathione S-transferase; HNE: 4-hydroxynonenal; LDL: low density lipoprotein; LH: lipid hydroperoxides; MDA: malondialdehyde; MNBCs: mononuclear blood cells; ORAC: Oxygen radical absorbance capacity; Ox-LDL: oxidized LDL; SOD: superoxide dismutase; PBMCs: peripheral blood mononuclear cells; QE: quercetin equivalents; SBs: strand breaks; TAC: total antioxidant capacity; TAS: total antioxidant status; TBARS: 2-thiobarbituric acid reactive substances; TE: Trolox equivalents; TEAC: Trolox equivalent antioxidant capacity; TRAP: total radical trapping antioxidant parameter.
REFERENCES


37 EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), Guidance on the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health. *EFSA J.*, 2011, **9**, 2474.


