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Short-term blueberry intake enhances biological antioxidant potential and modulates inflammation markers in overweight and obese children

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Abstract. Oxidative stress and inflammation together play a crucial role in the obesogenic process, and imbalances in reactive oxygen species, free radicals and antioxidants have been reported as being major mechanisms underlying obesity-related co-morbidities. Obesity and oxidative stress may be present even within the first two decades of life, and chronic exposure to systemic inflammation may contribute to the onset and progression of cardiovascular disease and diabetes. Bioactive compounds present in blueberry have been shown to have many positive effects on human health. The present study was carried out in northern Italy on a population of 24 overweight and obese children (8–13 years), divided into three groups: the first consumed fresh blueberries, the second blueberry purée, while a third control group did not consume any blueberries. The children's anthropometric measures were taken and serum markers related to inflammation, CRP, ceruloplasmin, and complements C3 and C4 were measured during the eight weeks they ate either fresh blueberries or blueberry purée. BAP test (Biological Antioxidant Potential) values of the three groups were monitored throughout the entire study and correlated with inflammatory, metabolic and anthropometric markers. The results showed a higher increase in antioxidant levels in the group that ate fresh berries than in the group that ate purée, while the control group's BAP values decreased over the eight weeks of the study. Our results show that increased consumption of blueberries, hence antioxidant intake, may also have a positive effect on markers of inflammation and oxidative stress in overweight and obese patients during childhood.

Keywords: Antioxidants, inflammation, obesity, blueberry

1. Introduction

Obesity in children is recognized as highly detrimental to health [37] and is a risk factor in several chronic diseases and disorders such as CVD, hypertension, type 2 diabetes, asthma and some malignancies [29]. An effective treatment of obesity, direct or indirect, may therefore offer protection against these diseases and reduce rates of morbidity and mortality. The International Obesity Taskforce (IOTF) estimates the global prevalence of school-aged children who are overweight or obese to be 10% [30]. In Italy, the mean percentage of children who are obese is around 12%, overweight about 24% (WHO data), although there is considerable regional variation with the south having higher

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rates than the north (2010 data for children aged 6–17 years from the Italian Ministry for Health, Okkio alla Salute Project). Fatness in children is likely to persist into adulthood and is the forerunner to obesity-linked pathologies and early mortality in adults [28].

While dietary reform remains the basis for weight control, other key factors include prevention of obesity during childhood, changes in the environment, team-working and participation in community development projects [5].

Oxidative stress and inflammation together play a crucial role in the obesogenic process while imbalances in reactive oxygen species (ROS), free radicals and antioxidants have been reported as being major mechanisms underlying obesity-related co-morbidities [23, 25]. Obesity and oxidative stress may be present in the first two decades of life [49] and chronic exposure to inflammation may contribute to the onset and progression of cardiovascular disease and diabetes. Various clinical interventions are available for decreasing oxidative stress in obese subjects and for maintaining normal cellular redox status, tissue function and intracellular signaling processes. Excessive ROSs have been reported as being involved in lipid impairment, protein and DNA damage and cell-function defects. Various obesity studies have found ROSs to be implicated in atherosclerosis, type 2 diabetes, arthritis [9, 22], and periodonotitis [5], while increased oxidative stress in accumulated fat is a significant pathogenic mechanism in obesity-related metabolic syndrome in mice and humans.

In particular, obesity causes an imbalance in the antioxidant defenses of various tissues: the primary reason is probably low dietary intake of antioxidants and phytochemicals which have antioxidant properties [50].

Vincent and Taylor [48] have identified and reviewed several factors that may give rise to oxidative stress in obesity: hyperglycemia, elevated tissue lipid levels, inadequate antioxidant defenses, chronic inflammation, excessive leukocyte infiltration and activation, endothelial ROS production, excessive hormone production by the renin–angiotensin system, and hyperleptinemia are all potential contributory factors. In each of these cases, either there is a direct increase in free radical production or there is insufficient antioxidant power to attenuate free radical damage. Some or all of these factors contributing to systemic oxidative stress may co-occur with obesity. Whether oxidative stress causes or intensifies disease in obesity is unclear, although several studies have reported a link between obesity and chronic low-grade inflammation. Furthermore, inflammatory pathways could be critical factors in the mechanisms underlying obesity and associated complications, although many questions remain unanswered, especially with respect to humans [41, 43].

Inflammation has been highlighted as playing a key role in the pathogenesis of cardiovascular diseases and many inflammatory markers have been identified. The inflammatory process seems to arise in the adipose tissue which participates in the regulatory process through cytokine production. Increased serum levels of tryglycerides, total cholesterol and LDL-cholesterol also play a role.

Inflammation of adipose tissue provokes insulin resistance and other complications typical of obesity, all of them associated and correlated with oxidative stress and adipocyte death.

Bioactive compounds in blueberry, mainly anthocyanins, have been shown to have many positive effects on human health, in particular in protecting the nervous system [45, 24]. Blueberry extracts have been found to have some effect in reversing decline in neural and cognitive functioning [3, 54], and to penetrate effectively cell membranes and provide antioxidant protection [20]. Anthocyanins are the most numerous class of compounds, followed by hydroxycinnamic acids, primarily trans-chlorogenic acid.

Although an increased intake of flavonoids – isoflavones, anthocyanidins, flavones and flavonols – in the ordinary diet has been reported to have many beneficial effects with respect to various pathologies in different age populations, there is no proof that high levels of antioxidant capacity in foods indicate their potential for altering antioxidant status *in vivo* [5, 38]. The antioxidant effect may be produced either directly or indirectly, largely depending on the bioavailability of the antioxidant compounds and how these affect cellular processes [42].

Human metabolism of flavonoids, along with absorption and excretion, are not clearly understood: flavonoids are absorbed from the intestinal tracts and are excreted either unchanged or as flavonoid metabolites in the urine or feces. Two other limiting factors concern measures taken after oral administration of pharmacological doses of individual flavonoids as opposed to dietary (food-based) levels of flavonoids, and the use of the aglycon form of flavonoids rather than the glycosylated flavonoids which predominate in plants. A study by Paganga et al. [35] on human serum *in vivo* showed that phloretin and quercetin are absorbed from the diet as glycosides. In a study on anthocyanins in red wine by Lapidot et al. [27], anthocyanin levels in the urine reached a peak within 6 h of consumption, although some compounds seemed to undergo substantial modification at the molecular level.

The average intake of anthocyanins by western populations is estimated to be 12 mg/day/person [52], assuming a daily energy intake of 2000 kcal.

Blueberry has recently been shown to be effective in reducing global upregulation of inflammatory genes and oxidative stress in hamsters, owing to its cytoprotective and anti-inflammatory properties [26]. DeFuria and colleagues [15] showed how blueberry can attenuate insulin resistance in mice as a consequence of adipocyte reduction and inflammatory status/sequelae. Earlier studies conducted on anthocyanins from blueberry reported them to be effective in preventing obesity, while whole berries were not [38]. Cyanidin 3-glucoside ameliorated insulin sensitivity and hyperglycemia in mice [40].

The present study was designed to examine whether the consumption of blueberry by obese children positively affects antioxidative balance and influences inflammation markers.

2. Materials and methods

2.1. Study population

The study was carried out in the Province of Trento, in northern Italy. The subjects were all under the supervision of the Nutrition Service and were following a hypocaloric diet. They were contacted by telephone and invited to participate as volunteers in the research. All the participants' parents were informed of the aims and procedure of the study and approved and signed a written consent, while the children were informed that they were free to withdraw from the study at any time. The 24 subjects were specifically chosen from a pre-adolescent age range of 8 to 13 years. Patients with additional pathologies were excluded. The study underwent prior evaluation and was accepted by the hospital's Ethics Committee. The study population was split into three groups with males and females randomly divided between them: one group was chosen as a control, one followed a diet integrated with 375 g/week of fresh blueberry fruit, and one group followed a diet integrated with 375 g/week of fresh puréed blueberry fruit. All subjects were matched for oral intake which was calculated by a registered dietitian on the basis of a 24 hour recall. The study covered a period of 8 weeks, starting on December 14, 2009 and ending on February 15, 2010. In the first session (December 14, 2009) the 24 subject-patients underwent a complete blood analysis and anthropometric measures; these procedures were repeated on January 12 and on February 15, 2010 at the Nutrition Service.

2.2. Children's anthropometric measures

Waist circumference (cm) was measured with a tape measure to the nearest 0.5 cm between the 12^{th} rib and the iliac crest by the same operator each time with the help of a recorder. Height was measured with a statometer (SECA Germany) to the nearest 0.5 cm, without shoes, eyes looking straight ahead and with a right-angled triangle resting on the head and against the wall. Weight was measured with a lever balance (SECA Germany) to the nearest 100 g, without shoes, in light undergarments. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Following Cacciari [7], overweight and obese were defined in terms of relative percentiles. Systolic and diastolic measures of the patients were taken in horizontal position. Skinfold thickness was measured at the triceps, biceps and subscapular sites using a skinfold caliper (Holtain Ltd. U.K.) by the same operator at each timepoint. All measurements were taken three times at each site on the right side of the body and then averaged. Fat mass was calculated following Slaughter et al. [44], with different criteria according to gender. Baseline variables of the three groups are reported in Table 1 .

2.3. Blood chemistry

Blood samples were collected from all subjects between 8:00 and 9:00 am at each timepoint after 12 hours of fasting and subjects were advised not to eat specific foods high in antioxidant content, a list of which was provided. Biochemical evaluation was carried out in the same laboratories for the entire duration of the experiment. Some of the blood samples were processed immediately and some were stored at -30°C for BAP tests. Venous blood samples

Table 1
Baseline variables of the study population

Variables	Control group Mean ± SD	BB fruit group Mean ± SD	BB purée group Mean ± SD
CRP (mg/L)	0.7 ± 0.6	2.2 ± 2.1	4.8 ± 6.8
Age (years)	10.2 ± 1.6	12.5 ± 1.3	11.6 ± 1.6
BMI percentile (a)	90.4 ± 7.3	94.8 ± 9.1	91.3 ± 8.5
Glucose (mg/dL)	90.3 ± 4.7	86.3 ± 4.4	94.4 ± 4.8
Total proteins (g/L)	74.8 ± 1.8	74.7 ± 2.6	76.1 ± 3.5
Albumin (g/L)	47.2 ± 1.7	46.7 ± 1.6	46.9 ± 2.0
Cholesterol	161.0 ± 33.1	151.7 ± 17.9	145.0 ± 30.8
HDL (mg/dL)	58.0 ± 10.5	54.3 ± 9.6	50.3 ± 9.9
LDL (mg/dL)	91.7 ± 35.0	85.2 ± 12.7	83.1 ± 26.2
Triglycerids (mg/dL)	71.7 ± 58.1	61.3 ± 25.8	72.0 ± 43.0
IgG (g/L)	10.8 ± 2.1	11.3 ± 1.7	11.9 ± 1.8
IgA (g/L)	1.6 ± 0.6	1.3 ± 0.6	1.3 ± 0.6
IgM (g/L)	1.0 ± 0.3	1.3 ± 0.7	1.2 ± 0.5
C3 (g/L)	1.1 ± 0.2	1.2 ± 0.2	1.4 ± 0.2
C4 (g/L)	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Transferrin (g/L)	2.7 ± 0.2	2.7 ± 0.3	2.8 ± 0.4
Ceruloplasmin (g/L)	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Pre-Albumin (g/L)	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
SP (mm/Hg)	111.6 ± 10.6	117.1 ± 7.1	115.3 ± 9.8
DP (mm/Hg)	59.7 ± 14.7	68.1 ± 8.6	69.6 ± 10.1
Waist (cm)	79.3 ± 3.7	91.4 ± 7.9	89.6 ± 5.3
Muac (cm)	28.2 ± 3.6	31.2 ± 4.8	30.0 ± 4.0
TS (mm)	25.8 ± 5.7	31.0 ± 5.3	30.4 ± 5.4
Wrist	15.8 ± 0.8	16.9 ± 0.9	16.0 ± 0.9
Fat Mass (b)	23.7 ± 4.4	26.8 ± 3.5	26.9 ± 4.2

Legend: BB: blueberry; SP: Systolic Pressure; DP: Diastolic Pressure; TS: Tricep skinfold; a) according to Cole and Cacciari; b) according to the Slaughter formula.

were centrifuged at 37°C and the recovered plasma specimens were stored immediately at −30°C until analysis. All the marker analyses conducted on the 24 obese children and a description of the methods used to monitor the markers at the different timepoints are summarized in Table 2 .

2.4. Determining children's oxidative balance

Serum oxidative balance was evaluated with a BAP test (Diacron International s.r.l.) carried out on centrifuged blood stored at −30°C until analysis. BAP tests were conducted with the OLYMPUS 450 Multiple Analyzer (Roche, Switzerland), while the anti-ROM test was done with FREE-CARPE DIEM equipment (Diacron International s.r.l., Grosseto, Italy). The biological antioxidant potential (BAP) is the plasma barrier component with oxidation reduction potential which provides reactive species with reducing equivalents. The BAP test is a means of assessing the antioxidant potential of blood plasma by measuring its ferric reducing ability. A sample of blood plasma is added to a colored solution of ferric ions (Fe^{3+}) bound to a special chromogenic substrate; the mixture decolors when its Fe^{3+} ions are reduced to ferrous ions (Fe^{2+}), the intensity of the chromatic change reflecting the ferric reducing - hence antioxidant - ability of the sample. The reduced iron concentration is photometrically assessed by comparing it with a standard serum and monitoring absorption over 505 nm at 37°C. The results are expressed in micromoles of reduced iron/liter using ascorbic acid as a standard [16, 36]. In the standard protocol, 50 microliters of a ferric solution are

Table 2
Description of the markers, units of expression, sample types and methods used

Description	Unit	Sample type	Official method and references
Insulin	μU/mL	snap-frozen serum sample	Enzyme-labeled chemiluminescent immunometric assay [6]
C Reactive Protein	mg/L	snap-frozen serum sample	PEG-enhanced immunoturbidimetric [33]
Immunoglobulin A	g/L	snap-frozen serum sample	PEG-enhanced immunoturbidimetric [33]
Immunoglobulin M	g/L	snap-frozen serum sample	PEG-enhanced immunoturbidimetric [33]
Erytro Sedimentation Rate	mm/h	whole blood	Capillary microphotometer [46]
HDL	mg/dL	snap-frozen serum sample	Elimination/Catalase [47]
LDL	mg/dL	snap-frozen serum sample	Elimination/Catalase [47]
Triglycerids	mg/dL	snap-frozen serum sample	GPO, Trinder without Serum Blank (50)
Immunoglobulin G	g/L	snap-frozen serum sample	PEG-enhanced immunoturbidimetric [33]
Cortisol	μg/dL	snap-frozen serum sample	Competitive chemiluminescent enzyme immunoassay [6]
Complement C3	g/L	snap-frozen serum sample	Nephelometry [11]
Glucose	mg/dL	snap-frozen serum sample	Hexokinase [12]
Albumin	g/L	snap-frozen serum sample	BCG Dye Binding [12]
Cholesterol	mg/dL	snap-frozen serum sample	Enzymatic [47]
Complement C4	g/L	snap-frozen serum sample	Nephelometry [11]
Transferrin	g/L	snap-frozen serum sample	Nephelometry [11]
Ceruloplasmin	g/L	snap-frozen serum sample	Nephelometry [11]
Pre-Albumin	g/L	snap-frozen serum sample	Nephelometry [11]
Total Proteins	g/L	snap-frozen serum sample	Biuret [12]
Glucagon	ng/L	snap-frozen serum sample	Radioimmunoassay [18]

diluted in 940 microliters of thyocinate solution producing a colored mixture, and the initial rate of absorption is determined with photometry. Immediately afterwards, 10 microliters of the sample serum are added and the solution is incubated at 37°C for 5 minutes. It is then photometrically assessed for a second time. Our study was the first time the procedure had been modified using an Olympus Roche multiplex analyzer, the quantities of serum and reagents proportionally adjusted to 300 microliters.

On the basis of large population studies [14], values of 2200 micromoles/L are considered normal while values below this threshold are considered pathological events. The test is linear from 500 to 8000 micromoles/L. The intra- and inter-variation coefficient scores in the experimental conditions were in all cases below 5.

The BAP test is derived from the FRAP assay [4] and gives a reliable measure of serum antioxidant capacity in terms of its iron-reducing activity, which mainly concerns a complex of substances involved in protecting against free radical attacks [8, 32].

2.5. Berry samples

A mixture of different varieties of highbush *V. corymbosum* blueberries were delivered every week to the children through the Nutrition Service in pre-prepared doses stored at +2°C–+4°C. Blueberry was chosen from among other berries because of its high antioxidant content and its very rare reported allergies. Berries, both fresh and puréed, were analyzed weekly in three replicates using the Folin-Ciocalteu protocol for total polyphenols and anthocyanin content; analyses were made at different times of the week in order to monitor for modifications during storage.

2.6. Statistical analysis

Continuous variables are presented as mean - standard deviation. Correlations between normally distributed continuous variables were assessed by calculating Pearson's partial r coefficient. The relationships between anthropometric measures and marker concentrations (dependent variable) within each group, with separate comparisons of the

Table 3
Blueberry nutritional and commercial characteristics

	Mean ± SD
Total polyphenols (mg/kg (+) catechin)	1906.6 ± 432.4
Total Anthocyanins (mg/kg malvidin 3-glucoside)	1312.5 ± 297.9
Resulting Total Anthocyanins (mg/portion)	65.6 ± 14.9
Single berry weight (g)	1.8 ± 0.4
Single berry height (mm)	11.6 ± 0.9
Single berry diameter (mm)	15.0 ± 1.6
Pedicel scar (mm)	2.2 ± 0.5
Calyx Scar (mm)	7.4 ± 1.3
Fruit firmness (Kg/cm^2)	0.1 ± 0.1
RSR ($Brix^\circ$)	11.8 ± 1.4
Titratable Acidity (meq/100 g)	28.9 ± 13.4

intermediate and final patient analyses, were investigated using a t-student test. All reported p values are based on two-sided tests. We obtained a statistical power of <0.05 (p value) probability for both the t-test and the correlations. A principal-component factor analysis was used to investigate the relationships between the correlated markers and risk factors. Statistica v.8 software was used for all the statistical calculations.

3. Results and discussion

3.1. Fruit samples

Berry characteristics are summarized in Table 3. The fruit were reported as having been imported to Italy from Mexico and Chile, and although they may not have been the most suitable fruit with respect to freshness and storage quality, their total anthocyanin content was nonetheless on average 5.47 times the adult physiological requirements of 12 mg/day given a daily energy intake of 2000 kcal. Comparison with locally produced fruit, based on analysis of the anthocyanin content of 24 different highbush blueberry cultivars grown over one season in 2008 in Trentino, is presented in Fig. 1. Most (19 out of 24) of the available commercial varieties analyzed had total anthocyanin values ranging between 1000 and 2000 mg/kg of malvidin 3-glucoside. The blueberries we used in this experiment had an average total anthocyanin content measured in malvidin 3-glucoside of 1312.5 mg/kg. The other quality parameters, morphological traits and organic values, were also comparable to most of the commercial varieties available daily on the market [21].

3.2. Antioxidative balance and inflammation in obese and overweight children

Evaluation of antioxidative balance was the main aim of this study, and we can draw a few significant conclusions in this regard. Free radicals (data not shown) were high at the starting point (t_0) of the trial in all three groups investigated, especially the group that consumed whole blueberry fruit, clearly indicating a pathological state.

The total oxidant capacity of blood plasma against N,N-diethylparaphenylendiamine was measured by means of a d-ROMs test (data here not shown), according to the method described by Alberti et al. [1]. We considered the obese children with the highest values of plasma oxidant capacity to be the best candidates for fresh fruit consumption and we subsequently found that their plasma antioxidant capacity (as measured with a BAP test) increased significantly after consuming fresh blueberry fruit (see below).

From initial high levels of oxidant species in the entire study population, there was a significant improvement in the BAP parameter at the end of the first month (t_1) in the group treated with blueberry fruit, indicating a significant increase ($p < 0.03$) in antioxidant capacity (Fig. 2). At the second timepoint the increase was not significant ($p = 0.06$),

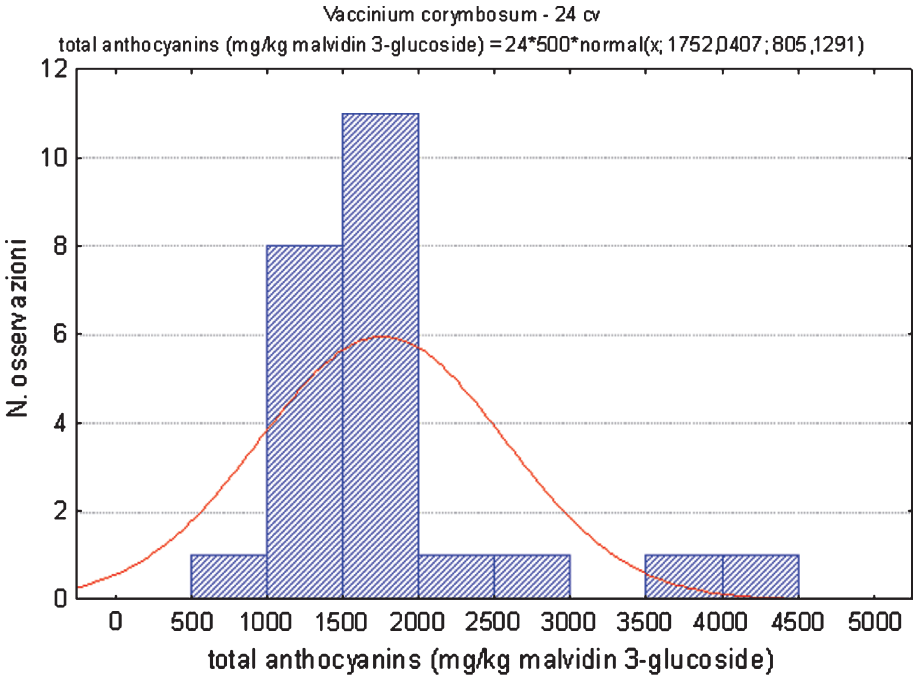

Fig. 1. Total anthocyanin content of 24 commercial cultivars grown in Trentino in 2008.

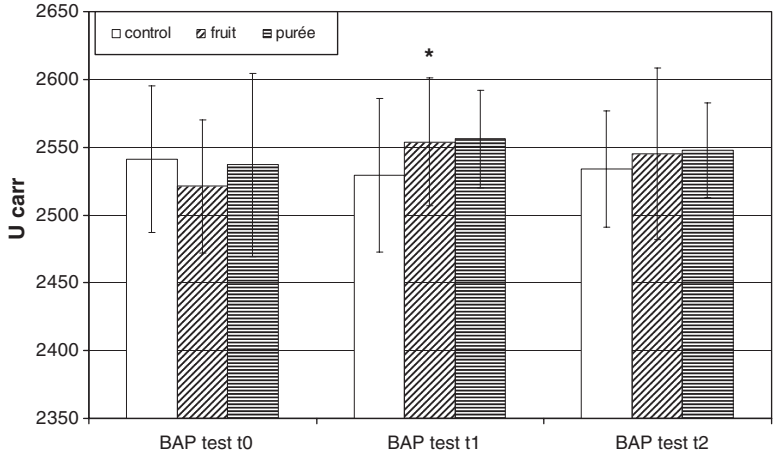

Fig. 2. BAP test results at the three timepoints in the control, whole fruit and purée intake groups.

although the trend remained positive until the end of the treatment. In fact, BAP levels in the group consuming fresh fruit increased until the eighth week, indicating that antioxidant capacity of the fruit provided enhanced, although transitory, protection. In the group treated with blueberry purée there was an increase in BAP, although not significant, over the 60 days of consumption, while the BAP values of the control group decreased over the eight weeks.

The results of the BAP tests seem to paint a picture in which antioxidative–oxidative balance is significantly influenced in the short term by blueberry consumption, with the clearest results seen in fresh fruit consumption after the first month. Of course, just one parameter, in this case bioactive compounds derived from blueberry, cannot

be expected to bring about long-term changes to a multifactorial situation caused by obesity, in which systemic homeostasis may well interfere with restabilization of the oxidant - antioxidant balance. However, the study confirms the positive effects of blueberry consumption as part of the diet and it would definitely be interesting to investigate the issue further.

Regarding the differences between the two treatments, the purée may be susceptible to oxidation processes caused by the preparation and the time between preparation and consumption. In addition, bioavailability and kinetic absorption of the antioxidant compounds may be different in the two forms of blueberry, fresh and puréed.

Studies on the relationships between inflammatory pathway markers, obesity status and antioxidant balance in children and adolescents are scarce. In this study, body fat measures, namely BMI percentiles, waist circumference, tricep skinfold and fat mass, were associated with acute phase inflammatory proteins – CRP, ceruloplasmin, C3 and C4 – and closely related to triglycerides, prealbumin and total proteins. In addition, markers of obesity status, such as hypercholesterolemia and high levels of LDL, are inversely associated with antioxidant potential. Correlation studies on the baseline traits of the entire study population revealed a significant inverse correlation between BAP and cholesterol ($r = -0.46$, $p = 0.02$) and LDL ($r = -0.56$, $p = 0.005$).

Total proteins were also correlated with biological antioxidant potential ($r = 0.46$, $p = 0.02$), triglycerides ($r = 0.41$, $p = 0.47$) and immunoglobulin G and A ($r = 0.50$, $p = 0.01$ and $r = 0.46$, $p = 0.02$, respectively).

Complement factor C3 is the precursor of a potent activator and chemotaxin of macrophages that play a role in inflammation. C3 is highly expressed in adipocytes and has previously been related to obesity and insulin resistance. Once macrophage activation and infiltration is underway, macrophage-mediated inflammatory response leads to impaired insulin response in adipocytes. In this study, BMI percentile also correlated with C3 ($r = 0.63$, $p = 0.001$) and C4 ($r = 0.55$ with $p = 0.006$). As a consequence, insulin signaling in adipocytes could become increasingly impaired, eventually leading to massive adipocyte lipolysis, necrosis and systemic insulin resistance [53]. Although most of the evidence was obtained from obese adults, some studies have also confirmed the involvement of C3 and C4 in obesity in children and adolescents [51].

Complementary factors 3 and 4 (C3, C4) are the major plasma proteins of the complement pathway of the immune system and synthesis of them increases in response to inflammation and infection. In our study population, complement components C3 and C4 both correlate with BAP ($r = 0.63$, $p < 0.001$ and $r = 0.54$, $p = 0.006$ respectively) and C3 also positively correlates with total proteins ($r = 0.69$, $p < 0.0001$) and with triglycerides (0.68, $p = 0.0001$), indicating an increase in the response to low chronic inflammation in these obese children and a risk of metabolic syndrome [31]. These kinds of changes in very young obese children are thought to predispose them to enhanced fat storage and decreased fat oxidation driving the obesity profile further [10]. An increase in C3, in particular, has been associated with weight gain, inflammation, atherosclerosis and the risk of cardiovascular disease [17].

BMI also positively correlated with another important inflammation marker, ceruloplasmin ($r = 0.90$, $p < 0.0001$). This protein acts as an antioxidant in serum by oxidizing ferrous ion which could otherwise act as a catalyst in free radical reactions, but in special conditions, such as low pH in tissue compartments with inflammation, where ceruloplasmin could act as a pro-oxidant through donation of free copper ions that generate free radicals and cause oxidative tissue damage or through LDL oxidation. Ceruloplasmin is also a positive acute phase protein, and an increase in serum concentration may provide information about inflammation processes in organisms [34], supported here by the slight inverse correlation with BAP values ($r = -0.3$). In this study, ceruloplasmin positively correlated with CRP values ($r = 0.52$, $p = 0.01$), C3 ($r = 0.48$, $p = 0.01$) and C4 ($r = 0.61$, $p = 0.002$), all factors highly predictive of cardiovascular events in adults.

The complete baseline Pearson correlation matrix at $p < 0.05$ is reported in Table 4.

In conclusion, one of the intervention strategies for lowering oxidative stress in obese children and thus reduce potential onset of morbidity is a diet which includes high antioxidant foods. Consumption of blueberry, the fresh fruit in particular, can play an important role in such a diet. Compared with other interventions, such as a low-calorie diet, regular exercise and supplements, we would also like to point out the palatability and satiety effects of fresh blueberry (data not shown).

Obesity is clearly related to impairment of inflammation biomarkers and antioxidant balance in childhood, and our study confirms other studies conducted mainly with adult populations. Correcting systemic oxidative stress, extenuated by low levels of antioxidants in obesity, may be a good strategy to adopt in the fight against endothelial dysfunction and insulin resistance.

Table 4
Significant Pearson Correlation values of the different parameters measured on the population ($N = 24$)

Parameters	Significant correlations						
TP	BAP 0,5 (a)						
Cho	BAP −0,5(a)	Alb 0,6(c)					
HDL	Alb 0,9(c)	Cho −0,5(b)					
LDL	BAP −0,6 (b)	Cho 0,9(c)					
TG	TP 0,4(a)	LDL 0,5(b)					
IgG	TP 0,5(a)						
IgA	TP 0,5(a)						
C3	TP 0,7(c)	TG 0,7(c)					
C4	C3 0,6(b)						
TF	C3 0,4(a)						
CP	CRP 0,5(a)	C3 0,5(a)	C4 0,6(b)				
PreA	TP 0,5(a)						
Cort	C4 0,5(b)	CP 0,5(a)					
Age	Cho −0,5(a)	LDL −0,7(c)					
BMI	TP 0,4(a)	TG 0,4(a)	C3 0,5(b)				
BMIp	TP 0,6(b)	TG 0,6(c)	C3 0,6(b)	C4 0,5(b)	CP 0,4(a)	PreA 0,4(a)	BMI 0,7(c)
SP	TG 0,4(a)	C3 0,6(a)	C4 0,6(b)	TF 0,5(a)	CP 0,3(a)	PreA 0,4(a)	
DP	IgA −0,5(a)	C3 0,4(a)	C4 0,4(a)	SP 0,6(b)			
W	IgM 0,5(b)	Age 0,5(a)	BMI 0,8(c)	BMIp 0,5(b)			
M	Age 0,5(a)	BMI 0,5(a)	W 0,6(b)				
TS	TP 0,4(a)	IgM 0,2(a)	BMI 0,8(c)	BMIp 0,6(b)	W 0,6(c)	M 0,5(b)	
Wr	Age 0,6(b)	BMI 0,7(c)	W 0,6(b)	M 0,6(b)	TS 0,6(b)		
FMS	TP 0,5(a)	IgG 0,5(a)	C3 0,5(a)	BMI 0,7(c)	BMIp 0,5(a)	W 0,6(b)	M 0,5(a)
FMS	TS 1,0(c)	Wr 0,6(b)					

Legend: CRP: C-Reactive Protein; BAP: biological antioxidant potential; Glu: glucose; TP: total proteins; Alb: Albumin; Cho: cholesterol; TG: tryglicerids; TF: transferring; CP: ceruloplasmin; PreA: prealbumin; Cort: Cortisol; BMI: body mass index; BMIp: BMI percentile; SP: systolic pressure; DP: diastolic pressure; W: waist; M: Muac; TS: tricep skinfold; Wr: wrist; FMS: fat mass (Slaughter).
(a): $p < 0.05$; (b): $p < 0.01$; (c): $p < 0.001$.

Blueberry is a palatable fruit and the participants in the study, apart from 2 of the 24 children, asked if they could continue the experiment. This was in part because they enjoyed eating the fruit, but was also in large part, we believe, because of the positive effects of a favorable and communicative environment for the children, where their obesity-related problems could be aired and where they were given constant, additional motivation to reduce weight, change habits and maintain healthy behavior, particularly with regard to fruit consumption and food choices.

Eight weeks of treatment may not be sufficient to produce a statistically significant reduction or increase in a particular marker. In addition, most of the antioxidant compounds present in blueberries have a fast absorption and excretion rate, so that consumption over a longer period would probably be much more beneficial. Precise kinetic profiles should also be further developed.

This was a limited, exploratory study, conducted with only 24 children, and it needs to be extended to a larger sample. The dietary intake data are not very accurate and there are considerable inter-individual differences. Furthermore, since two children, both belonging to the control group, left the program after the first evaluation at t_0, the mean age of this group was lower than the other two. On the other hand, the other two groups were approaching puberty and we expect their antioxidant balance to be more compromised than earlier in childhood.

However, this study showed an increase in antioxidant levels together with a decrease in oxidant species, combined with a slight reduction in some of the clinical and chronic inflammation markers investigated; these effects were greater on average in the group that ate fresh berries than the group that ate the purée.

Our results show that increased consumption of blueberries, and therefore antioxidant intake, may also have an effect on markers of inflammation and oxidative stress in overweight and obese patients during childhood.

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