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Dried apples enriched with mandarin juice by vacuum impregnation improve antioxidant capacity and decrease inflammation in obese children

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Abstract

Background: **A favorable effect over development of degenerative diseases is derived of an adecuate intake of fruit and vegetables, mainly due to their antioxidant compounds**

Objectives: **The goal of this study was to test the effect in vivo over oxidant status and inflammation in obese children of a novel food product made of dried apples enriched with mandarin juice by vacuum impregnation.**

Methods: **A four-week intervention study was conducted in 41 obese children (> 2 standard deviation score-body mass index). Participants were instructed to follow their usual diet supplemented with 40 g/day of the developed product. Anthropometric parameters were determined including body mass index, waist circumference and estimations of body fat percentage using bioelectrical impedance. Dietary intake was assessed by questionnaire. Metabolic risk factors (blood pressure, lipid profile, glucose and insulin resistance) were recorded. To determine oxidant status, plasma total antioxidant capacity and 8-hydroxydeoxyguanosine, as marker of oxidative damage to DNA, were investigated. High-sensitive C-reactive protein, tumor necrosis factor-α, and interleukins 6 and 1-α were measured as inflammatory markers. Measurements were collected at baseline and at the end of the intervention period.**

Results: **Significant improvement in systolic blood pressure and lipid profile after intervention period was noted. A significant increase in the antioxidant capacity of plasma (ABTS and FRAP assays) and reductions in DNA oxidative damage and inflammatory markers were also found.**

Conclusion: **Overall, adding the product to the diet contributes to ameliorate oxidant and inflammatory status in obese children and several risk factors for atherosclerosis.**

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Key words: *Antioxidants. Cardiovascular risk. Children. Fruits. Obesity.*

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LA INGESTIÓN DE UN PRODUCTO DERIVADO DE MANZANA DESHIDRATADA ENRIQUECIDO CON ZUMO DE MANDARINA MEDIANTE TECNOLOGÍA DE IMPREGNACIÓN AL VACÍO MEJORA LA CAPACIDAD ANTIOXIDANTE Y DISMINUYE LA INFLAMACIÓN EN LOS NIÑOS OBESOS

Resumen

Antecedentes: **Una adecuada ingesta de vegetales previene el desarrollo de enfermedades degenerativas, principalmente debido a sus compuestos antioxidantes.**

Objetivo: **Evaluamos el efecto in vivo en los niños obesos de un nuevo producto alimenticio hecho de manzanas deshidratadas enriquecidas con zumo de mandarina mediante impregnación a vacío.**

Métodos: **Estudio prospectivo longitudinal de cuatro semanas de duración. Se estudiaron 41 niños obesos que suplementaron su dieta habitual con 40 g/día del producto desarrollado. Se determinaron parámetros antropométricos (índice de masa corporal, circunferencia de la cintura) y estimación de la de grasa corporal con impedancia bioeléctrica. La ingesta dietética se evaluó por cuestionario. Se registraron factores de riesgo metabólico (presión sanguínea, perfil lipídico, glucosa y resistencia insulínica). El estado oxidante se investigó mediante la capacidad antioxidante total del plasma y la 8-hydroxideoxiguanosina (marcador de daño oxidativo al ADN) y como marcadores de inflamación valoramos la proteína C-reactiva ultrasensible, el factor de necrosis tumoral-α y las interleukinas 6 y 1-α. Las mediciones se recogieron al inicio y al final del período de intervención.**

Resultados: **Encontramos una mejoría significativa en la presión arterial sistólica y en el perfil lipídico después del período de intervención. Igualmente demostramos un aumento significativo de la capacidad antioxidante del plasma, una reducción del daño oxidativo del ADN y de los marcadores inflamatorios.**

Conclusión: **La adición a la dieta del producto elaborado con manzana deshidratada, y enriquecido con zumo de mandarina mediante impregnación al vacío, contribuye a mejorar el estado oxidante e inflamatorio en los niños obesos, así como diversos factores de riesgo cardiometabólico.**

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Palabras clave: *Antioxidantes. Riesgo cardiovascular. Niños. Frutas. Obesidad.*

Abbreviations

ABTS: 2,2 -azinobis (3-ethylbenzothiazoline-6-sulfonic acid).
FRAP: Ferric reducing antioxidant power.
HDL: High-density lipoprotein.
IL-1α: Interleukin 1α.
IL-6: Interleukin 6.
8-OHdG: 8-hydroxydeoxyguanosine.
TNF-α: Tumor necrosis factor α.
VI: Vacuum impregnation.

Introduction

A large body of evidence indicates that the risk of degenerative diseases such as obesity, atherosclerosis, diabetes and cancer is considerably lower in people who consume above average amount of fruits and vegetables.[1] Various plant-derived compounds such as fiber or antioxidants including vitamins and other phytochemicals (polyphenols) may account for these protective effects.[2] Widely recognized as the best approach to ensure an adequate intake of antioxidant nutrients is the consumption of at least 5 servings of fruits and vegetables a day.[3] However this is not always a practical recommendation because of variations in individual dietary practices or preferences. In fact, specific studies carried out in children shown that few children meet these recommendations.[4] Given these facts, the intake of foods containing enhanced levels of bioactive compounds may be an ideal way to meet the recommendations with minimal intake.

The increasing public interest in the consumption of foods that have beneficial effects on health has motivated the food industry towards develop such products through efforts in both research and production. Apples are a major source of dietary antioxidants including phenolic compounds. Furthermore, apples can be used as a source for the production of healthy products. Other fruits rich in antioxidant compounds and vitamin C are citrus fruits. In previous studies we found that intake of mandarin juice, a product high in specific flavonoids and carotenoids[5] exert a beneficial effect on oxidative status of hypercholesterolemic[6] and obese children[7] that can prevent or delay the development of comorbidities.

Vacuum impregnation (VI) is a new technology used to introduce compounds into the internal structure of fruits and vegetables. One application of VI is to use fruit juices to produce fortified or value-added fruit products.[8] For example, VI can be used to introduce mandarin juice into apples, so that the apple matrix becomes fortified with ascorbic acid and flavonoids solely present in citrus fruits that possess high antiradical potential.[9] The food matrix provided by apples can serve to deliver bioactive substances at a level more concentrated than what is originally present in these fruits and can allow for the cooperative interaction of antioxidant nutrients. Moreover, the matrix may protect against oxidation of the compounds included. Thus, the VI technology can provide alternative products to increase the intake of physiologically active substances.

Obesity is a known oxidative and inflammatory condition.[10] The aim of the present study was to evaluate the effect of incorporating snacks made of dried apple enriched with mandarin juice by VI into the energy-restricted diets of obese children. Specifically the goal was to provide useful information about antioxidant and anti-inflammatory effects of the product *in vivo*. To this end, we conducted an intervention study to examine whether the dietary intake of the snack is associated with a modification of oxidative status and biomarkers of systemic inflammation in obese children.

Materials and methods

Subjects

The study was conducted over 4 weeks in 48 obese children (24 boys) aged between 9 to 15 years who had been recruited at the outpatient Pediatric Gastroenterology and Nutrition Clinic of Dr. Peset University Hospital where they were referred by their primary care pediatrician for diagnosis and treatment of obesity. All children were free of infectious and inflammatory illnesses, as was determined by clinical history. Inclusion criteria were body mass index z-score ≥ 2 standard deviations according to the Spanish cards. Exclusion criteria were secondary obesity, weight change (± 3 kg) or vigorous exercise within three months before the start of the study, use of vitamin supplements or refusal to take the product. The children were asked to follow their slightly energy-restricted diet corresponding to a 20% reduction of caloric intake with respect to the individual's energy expenditure as calculated by the Harrris-Benedict equation and applying the WHO's correction factors for physical activity. The physical activity pattern was evaluated by assigning to each activity the metabolic equivalent.

At the onset of the study the children underwent medical examinations that included anthropometry [weight, height, waist and hip circumferences and fat mass percentage by a bioelectrical impedance instrument with eight-contact electrodes (BC-418MA; Tanita Europe BV, Hoofddorp, The Netherlands)], measurement of arterial blood pressure and pubertal status evaluation. A blood sample was obtained to assay biochemical parameters and markers of oxidative status and inflammation which were performed in the Clinical Analytical and Research Laboratories of the hospital. Participants were instructed not to alter their habitual diet or physical activity level during the 4 weeks of the intervention period. The duration of four weeks was established based on data from our previous studies made with mandarin juice in which a change in antioxidant status was observed.[6,7]

Macronutrient and micronutrient intake during the intervention period was assessed. The subject's parents recorded what and how much the children ate and drank for three days on a food record. To follow the dietary intake, the parents were interviewed by a nutritionist at baseline and at two-week intervals during the intervention period. At the same time, they were given a 2-week supply of the product (slices of dried apple which can be consumed as snacks packaged in a bag of 40 g) and they were instructed to give it in two takes (breakfast and lunch) along with their regular food. The parents were encouraged to ensure that their child took the 40 g/day and not to modify their overall diet. A dietary record corresponding to the last three days of the 28 day period was collected and compared with baseline and intermediate records. Dietary records were analyzed using the software Alimentación y Salud, BitASDE (General Médica Farmacéutica, Granada, Spain).

After the 28 day supplement period, the subjects returned to the clinic to have anthropometric measurements taken and to give their second blood sample. Blood samples and urine from fasting subjects were collected at baseline and at the endpoint and were analyzed in a blinded fashion.

The study followed the Helsinki guidelines and all participants and their parents gave their written, informed consent to participate. The protocol was approved by the Ethics Committee of Dr. Peset University Hospital.

Fruit-based functional product

The product used in this research study was specifically designed for this study and it is not a commercial product. The specifications and the product development process have been published in a previous study.[9]

The fruit-based functional product was made solely from fresh apples and mandarin juice, without any further additives. Apples (cv. *Granny Smith)* were obtained from a local market. Peeled apples were cut into disc-shaped samples (5 mm thick, with a 65 mm external diameter and 20 mm internal diameter) following their vertical axes. Mandarin (cv. *Ortanique)* fruits were harvested in an orchard located in Turís (Valencia), Spain, and squeezed immediately for juice preparation. The fruits were washed by immersion in tap water, after which they were drained, and squeezed in an industrial extractor with finger cups (Exzel, Luzzysa; El Puig, Valencia, Spain). Raw juice was divided into two fractions and the low-pulp juice fraction was homogenized with a Manton-Gaulin pilot homogenizer (model 15M8TBA) at 15 MPa, centrifuged with a Westfalia centrifuge (model SAOH 205), and pasteurized at 63 ºC for 15 seconds.[11]

Table I
Physicochemical characteristics and composition of the dried apple snack enriched with mandarin juice

Energy (kcal/gsample)	0.55 ± 0.02
Water activity (aw)	0.501 ± 0.002
Soluble solids (g/gsample)	0.126 ± 0.150
Carbohydrates (g/gsample)	0.77 ± 0.05
Vitamin C (µg/gsample)	16.56 ± 2.03
Narirutin (µg/gsample)	40.4 ± 5.0
Hesperidin (µg/gsample)	124.3 ± 15.4
Didymin (µg/gsample)	15.3 ± 1.9
Total carotenoids (µg/gsample)	0.647 ± 0.390
Antiradical activity (At) (mgascorbic acid/mgsample)	1.86 ± 0.03

Data are means ± SD (n = 3)

The fresh apple slices were impregnated with mandarin juice on a pilot scale using equipment designed in the Institute of Food Engineering for Development of the Polytechnic University in Valencia, Spain, by employing a vacuum technology for impregnation developed by our group.[12] A vacuum pressure of 50 mbar was applied. Each apple slice was impregnated 20% v/v. Physicochemical characteristics (water activity and soluble solids content) and functional properties (antiradical activity and flavonoids content) of the snack were determined following the procedure described in Betoret et al.[9] Vitamin C was analyzed by HPLC.[13] All the values of the product developed and used to carry out the *in vivo* study had the same physicochemical and functional properties than those obtained by Betoret et al.,[9] and are presented in table I. The physiologically active compounds from mandarin juice were identified, confirming the introduction of mandarin juice into the porous structure of apple. Moreover, the three main flavonoids from mandarin juice (narirutin, hesperidin and didymin) were estimated in the final product so that 40 grams of dried product can provide the same amount of flavonoids as 500 ml of fresh mandarin juice.

After the manufacturing process, the snacks were packaged into portions of 40 g. The bags used were acquired from Swiss Pack® and had been designed with opaque material to reduce moisture gain and oxidation of the product. The bags were totally sealed to maintain freshness and were provided to the patients in 2-week supplies.

Blood samples

Blood samples were centrifuged at 3,500 g for 20 minutes to obtain cell pellets and plasma. Aliquots were frozen at -80 ºC in the first hour after collection and stored in Eppendorf tubes until they were processed. Serum glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein cholesterol, triglycerides, uric acid, and alanine aminotransferase were measured using direct methods (Aeroset System®). Insulin, homocysteine, and folic acid were measured using the automatyzed electrochemoluminescence immunoassay (Architect c8000®). Both systems (Architect c8000® and the Aeroset System®) were obtained from Abbott Clinical Chemistry (Wiesbaden, Germany). Apolipoproteins A1 and B were measured using kinetic nephelometry (Immage® Beckman Coulter Inc., La Brea, California, USA).

Antioxidant status and oxidative damage

Total plasma antioxidant capacity was determined using two methods. 1) The ferric reducing antioxidant power (FRAP) method of Benzie and Strain;[14] this method measures the increasing absorbance occurring at 593 nm due to the formation of tripyridyl-s-trizine complexes with ferric (II) in the presence of a reductive agent; 2) The 2,2 -azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization assay according the procedure of Re et al.,[15] which is based on the reduction of ABTS+• radicals by antioxidants in plant products.

For the quantification of 8-hydroxydeoxyguanosine (8-OHdG) levels in DNA, a marker of oxidative DNA damage we used urine samples. Analysis of 8-OHdG was conducted according to the kit protocol EIA Kit Cayman Chemical Item Number 589320 (Ann Arbor, MI, USA).

Inflammatory markers assay

High-sensitivity C-reactive protein (hs-CRP) was measured by nephelometry (Immage Nephelometer®, Beckman Coulter Inc., La Brea, California, USA). Interleukin 6 (IL-6), interleukin 1 (IL-1α) and tumor necrosis factor α (TNF-α) were determined in plasma using the Diaclone Research ELISA kits (IL-6 ELISpot Set catalogue Nº 856.021.001, IL-1α ELISA Set catalogue Nº 851.600.001, TNF-α ELISpot Set catalogue Nº 856.041.001) (Diaclone Research, Besancon, France) according to the manufacturer's instructions.

Statistical analysis

Sample size was calculated by considering TNF-α as the main variable and applying our previous values for the standard deviation of this marker.[16] We assumed that an arbitrary difference of 0.5 would be clinically relevant. The power of rejecting the null hypothesis was calculated at 80%. The sample size required was a minimum of 36 patients. The paired Student t-test was applied to detect differences before and after nutritional intervention. Each child served as the control of himself. All reported p values are for a two-tailed t-test, with statistical significance set at $P < 0.05$.

Results

A total of 41 subjects (20 boys and 21 girls, 11.8 ± 2.8 years of age) of the 48 recruited fully completed the study; thus, the reported compliance was 85%. There were no significant differences between nutrient intakes during the treatment period. During the study there was not a reduction in energy or nutrient intake whereas an increase in vitamin C intake was noted. All the children presented low consumption values (≤ 2 portions) of fruits and vegetables before intervention that was obviously increased in one portion after intervention. There were no other substantial modifications with respect dairy products, bread, meat or fish. The results from the anthropometric measurements and the clinical, dietetic and biochemical assessments are listed in table II. Incorporating the snack in the diet did not

Table II
Anthropometric, dietetic and biochemical data from the 41 obese children at baseline and after supplementation with the dried apple snack enriched with mandarin juice

	Baseline	*Endpoint*	*P-value*
Weight (kg)	67 ±17	67 ± 18	0.845
Height (cm)	156 ± 16	157 ± 16	0.040
Body mass index (kg/m²)	26.8 ± 3.5	26.5 ± 3.9	0.082
Waist circumference (cm)	90 ±12	90 ± 12	0.596
Impedance index (cm²/Ω)	40 ± 11	40 ± 10	0.610
Fat mass index (kg/m²)	10.0 ± 2.8	10.1 ± 3.2	0.488
Energy intake (MJ/day)	9.87 ± 1.85	9.81 ± 2.16	0.826
Energy from fat (%)	38.3 ± 4.6	37.2 ± 4.9	0.289
Energy from protein (%)	17.1 ± 3.4	18.0 ± 3.3	0.122
Energy from carbohydrates (%)	45.5 ± 5.6	46.6 ± 6.2	0.406
Vitamin C intake (mg/day)	74.7 ± 30.8	84.5 ± 41.2	0.010
Systolic blood pressure (mm Hg)	118 ± 9	115 ± 11	0.030
Diastolic blood pressure (mm Hg)	69 ± 10	67 ± 8	0.146
Insulin (μIU/mL)	17.6 ± 10.3	17.6 ± 11.8	0.974
Glucose (mg/dL)	94 ± 8	92 ± 8	0.071
HOMA-IR	4.0 ± 2.3	3.9 ± 2.7	0.833
Total cholesterol (mg/dL)	161 ± 35	160 ± 29	0.698
HDL-cholesterol (mg/dL)	43 ± 9	45 ± 8	0.024
Triglycerides (mg/dL)	103 ± 53	92 ± 40	0.046
Apolipoprotein A1 (mg/dL)	128 ± 20	135 ± 22	0.020
Apolipoprotein B (mg/dL)	77 ± 22	73 ± 19	0.042
Uric acid (mg/dL)	4.4 ± 1.3	4.4 ± 1.2	0.917
Folic acid (ng/mL)	7.6 ± 3.9	7.7 ± 4.5	0.959
Homocysteine (μmol/L)	8.3 ± 3.0	8.4 ± 3.3	0.559

HOMA-IR: Homeostasis model assessment of insulin resistance; HD: High-density lipoprotein.
Data are means ± SD.

Table III
Antioxidant capacity, oxidative damage to DNA, and inflammatory markers in the 41 obese children at baseline and after supplementation with the dried apple snack enriched with mandarin juice

	Baseline	*Endpoint*	*P-value*
FRAP(mmol/L)	1.12 ± 0.26	1.23 ± 0.29	0.025
ABTS (mmol/L)	2.60 ± 1.00	2.88 ±1.07	0.035
Urinary 8-OHdG (ng/mL)	28.1 ± 4.3	25.1 ± 3.7	0.030
Interleukin 1α (pg/mL)	3.04 ± 0.21	2.93 ± 0.16	0.044
Interleukin 6 (pg/mL)	7.1 ± 2.2	6.5 ± 2.3	0.028
Tumor necrosis factor α (pg/mL)	26.6 ± 5.3	23.4 ± 7.0	0.017
High-sensitivity C-reactive protein (mg/L)	2.86 ± 4.31	1.64 ± 2.96	0.060

FRAP: ferric reducing antioxidant power; ABTS: 2,2 -azinobis (3-ethylbenzothiazoline-6-sulfonic acid).
Data are means ± SD.

induce weight loss or promote waist circumference or fat mass reduction. However, an improvement in systolic blood pressure, lipid markers (increase in HDL-cholesterol and apolipoprotein A1 and decrease in triglycerides and apolipoprotein B) were noted. Noticeably these changes were mainly found in the children with more altered values, particularly of triglycerides and apolipoprotein B. Thus, triglyceride levels fell 23% in children with baseline values ≥ 110 mg/dL and only 8% in children with values below this amount. The reduction in apolipoprotein B was less pronounced because almost all baseline values were normal. However, in the children with values above 100 mg/dL were decreased by 11%, while in the children with values below 100 mg/dL only were decreased by 1%.

We assessed the effects of adding to the diet the apple snack enriched with mandarin juice on oxidative status of the obese children. The comparisons between antioxidant capacity and 8-OHdG before and after nutritional intervention are shown in table III. A significant and comparable increase in the antioxidant capacity of serum, measured by ABTS and FRAP assays, was found in relation to the baseline values. Indeed, the antioxidant activity statistically increased after the intervention period. We then analyzed the effect on DNA damage. The results show a significant reduction in the modified base 8-OHdG. Overall, these results show a decrease in oxidative stress in the children after intake of the snack.

Interestingly, the inflammatory markers TNF-α, IL-1α and IL-6 also decreased after the intervention period with respect to baseline values (table III). These results cannot be attributed to weight reduction, caloric deprivation or modification of lifestyle because the children did not modify these parameters during the intervention. Likewise, there was no difference between percentage of energy provided by macronutrients before and after intervention nor in the intake of different groups of foods.

Discussion

The main objective of this study was to investigate whether the addition to the diet of a product made of dehydrated apple enriched with mandarin juice by VI would affect oxidative status and inflammatory markers in obese children. Our study indicates that short-term intake (4 weeks) of the snack along with a calorie restrictive diet contributes to beneficial effects on the antioxidant capacity of plasma, inflammatory markers and other cardiovascular risk factors.

Citrus juices and apples, which are commonly consumed worldwide, account for a high ratio amount of bioactive compounds in diet.[17] Some of their components, including agents protecting against oxidative damage such as vitamins and phenolics, have received a great deal of attention as potential strategies for the prevention of chronic diseases. Consumption of citrus juices affects the concentration of vitamin C in blood as well as biomarkers of antioxidant status.[6] These juices also act against a number of diseases, such as coronary insufficiency[18] and some age-related disorders,[19] and even they may reduce the risk of some cancers.[20] Similarly, a high apple intake has been linked with decreased risk of cardiovascular disease, diabetes and cancers; these effects have been attributed to its content in flavonoids and fiber.[21] These fruits together other dietary compounds can fulfill the needs of antioxidants. However, the children often do not meet with the general recommendation of five portions of fruits and vegetables per day.

Fruit-derived products are an emerging subsection of functional foods that may be consumed for health improvement. Moreover, consumption of combined foods may exert more benefits that active components alone because the array of nutrients provided may have a synergistic effect. Thus, discovering new combinations of natural substances would be an important step. VI technology allows the introduction of mandarin juice into the structural porous matrix of apples. The application of a homogenization operation to citrus juices decreases the particle size and increases the stability of the functional compounds present in the cloud of the juice.[11] This technology allowed developing a functional food from apple (cv. *Granny Smith)* and homogenized mandarin juice (cv. *Ortanique)* in which functional properties has been quantified.[9] The antiradical activity of the developed product was determined resulting in higher antiradical activities than the fresh apple. Furtehrmore, the development of a functional fruit-based product that combines apples with mandarin juice is very interesting for several reasons. It could combine the beneficial effects of both sources into one food item, or even enhance the beneficial components in the juice by protecting them through inclusion into a food structure. Some recent studies have shown the antioxidant power of products made with different vegetable matrices such as fruit chips consisting of dried fruits or vegetables.[22] However, we did not find any studies to see the *in vivo* antioxidant effects of a product made with a vegetable matrix impregnated with fruit juice.

Because of the effects of fiber on satiety, apple dried could have a role in reducing the prevalence of overweight and obesity. Mandarin juice also has effects on obese children.[7] In our study, weight, waist circumference and impedance index was essentially unchanged from baseline. However, a slight reduction in BMI of 0.3 units was noted due to continued growth in height. This is the specific target for the treatment of obesity in children: the maintenance of body weight, while the height is increasing.

Altered lipoprotein profiles are also important factors in cardiovascular risk. Moreover, an increase in HDL-cholesterol plays a key role in the prevention of cardiovascular disease. We observed an increase in HDL-cholesterol and apolipoprotein A1 and a decrease in triglycerides and apolipoprotein B in the obese children in this study. It is of note that children who have more elevated values of triglycerides and apolipoprotein B presented a greater decrease. This finding emphasizes the protective effect with regard to cardiovascular risk. Previously it has been shown that citrus juice increases free cholesterol transfer to HDL and decreases triglyceride transfer to HDL,[23] and that a high daily intake of citrus juice (750 mL/day of orange juice) may have a beneficial effect on lipoprotein profiles.[24] However, it is difficult to ensure compliance with these large amounts in children. Apples, in addition, have lipid-lowering effects mainly attributed to their fiber[25] and polyphenol content.[26] In the product studied there could be a synergy between the flavonoids specific to the apples (quercetin) and the flavonoids specific to the mandarin juice (flavanones and polymetoxyflavones)[5] and vitamin C. This synergistic blend of plant compounds may modify lipid profiles and reduce the cardiometabolic risk by modulating oxidant and inflammatory processes.

The novelty of our study lies on the finding that the compounds present in the product can decrease some cardiovascular risk factors in obese children and act *in vivo* as antioxidants, lowering the inflammatory response. The impregnation of apple slices with mandarin juice resulted in increased antiradical capacities compared to apples without impregnation.[9] Indeed, we found improved oxidative status in the subjects after the intake of the product which suggests that the bioavailability of its compounds produces an attenuation of oxidative injury.

Obesity is associated with a long-term inflammatory condition[27] that is linked to premature atherosclerosis. The major effect observed in our study was the reduction in plasma TNF-α, IL-1α and IL-6 concentrations within a short period of intake of the apple snack enriched with mandarin juice. The reduction in markers of inflammation and oxidative stress in obese subjects may offer an alternate strategy to improve the factors involved in the early onset of arterial damage.

The use of antioxidants that naturally occur in whole foods, instead of synthetic antioxidants that may have potential toxicities, is now in high demand.[28] In this sense, one possible way to improve the quality of the diet is through the supply of fruit and vegetable snacks.[29] One potential controversy is that snacking has been linked to an increase in total energy intake, particularly in adolescents.[30] In general, snacks can promote obesity because they are often high-fat and high-sugar foods. Given that salty snacks and chips are a very popular part of the diet of young people, we sought to replace them with other products that possess similar sensory properties (e.g. crispness, thickness), but carry significantly higher amounts of potentially bioactive compounds. Healthy snacks will be more easily accepted if their sensory characteristics of palatability and consistency are adequate to children. In this sense, the snack made of died apple impregnated with mandarin juice offers a plausible alternative to other harmful snacks.

The present study has limitations, including the relatively low number of subjects studied. It is possible that, if the children would have followed a recommended intake of fruits and vegetables, the effects would be similar. We have not made a comparison with children eating only dried apple. However, our specific goal was evaluate the effects *in vivo* of the product previously analyzed *in vitro* and we undertaken a well-characterized cohort studied by a meticulous longitudinal dietary analysis. One other possible limitation could be that the effects could be due to changes in macronutrient intake, such as fat and carbohydrates induced by the intervention. However, children followed the same type of diet before and during supplementation. Moreover, the analysis of the food records indicated that the macronutrient intakes were similar at baseline and endpoint. This suggests that the observed changes in oxidant status and inflammatory markers were specifically related to the snack intake.

Incorporation of a product made of dried apple enriched with mandarin juice to the hypocaloric diet in children will increase healthy bioactive compounds and also could decrease the intake of snacks with added fat and sugar calories. Both effects can help to counteract the deleterious consequences of childhood obesity.

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