Vadalà et al. Int J Sports Exerc Med 2016, 2:046
Volume 2 | Issue 4
ISSN: 2469-5718

International Journal of
Sports and Exercise Medicine

Research Article: Open Access

Energy Restoration by an Original Fruits & Vegetables Juice Intake in a Cohort of Elderly People Affected by Sarcopenia

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Abstract

Objective: To assess the efficacy of fruits & vegetables juice consumption on daily energy balance in a homogenous cohort of sarcopenic old patients.

Methods: 30 participants (27 women and 3 men) affected by sarcopenia were prescribed to drink a fixed volume of fruits & vegetables juice for 3 months. In this anecdotal and retrospective observational study, we filled questionnaire (0-5 rating) describing subjective well-being feelings, psycho-neurological traits, gastrointestinal, musculoskeletal, and cardiovascular as well as any untoward effects. Muscle strength and antioxidant saliva status were the parameters investigated by means of a non-invasive and practical hand dynamometer "FH-500" (Rupac Srl, Milan, Italy) and Point of Care FRAS 4 Evolvo SAT Test (H&D, Parma, Italy), respectively.

Data were statistically analyzed by Mann-Whitney test (continuous variables not normally distributed) and chi squared test (categorical variables).

Results: All parametric data (body weight and BMI) were not significantly different between 1-day and 90-day average for all the patients. On the contrary, significant improvement ($p < 0.02$) between 1 and 90-day based on the clinical questionnaire were found in the all the patients: specifically, increased ratings for energy level, athletic performance, quality of sleep, ease of awakening and spontaneous sleep induction, ability to focus on activities, mental acuity. The administered juice reduced significantly also asthenia and stress. No significant changes in gastrointestinal, musculoskeletal or cardiovascular system were observed. The main isometric strength values, as well as the antioxidant saliva status, were increased in all the patients at the end of the treatment ($p < 0.03$).

Conclusions: The results definitely showed that daily consumption of fruits & vegetables juice for 90-days increases the subjective feelings of general well-being, and improves some neurological/psychological performances. The protocol had very high performance compliance and no drop out, supporting the hypothesis of a possible prescription even in the clinical practice.

Keywords

Antioxidant, Energetic, Fruit, Vegetable, Juice, Sarcopenia

Introduction

In the recent years, several epidemiological studies showed that a high intake of fruits and vegetables is associated with a decreased risk of chronic diseases, such as cardiovascular, neurodegenerative and cancer [1-8]. This benefit has been mainly related to the antioxidant activity of the bioactive components of the foods, including Vitamin C, E, carotenoids, and phenolic compounds (e.g. flavonoids) that quench free radicals and reduce oxidative damage to cell structures, and DNA [9-11].

Vitamin C (ascorbic acid) is a water-soluble free radical scavenger, that reduces the risk of arteriosclerosis, cardiovascular diseases and cancer [12,13]. Moreover, it regenerates vitamin E in cell membranes in combination with Glutathione (GSH) or compounds capable of donating reducing equivalents [14,15]. Vitamin E (α-tocopherol) acts as 'chain breaker' during lipid peroxidation in cell membranes and various lipid particles including low-density lipoprotein (LDL). It blocks lipid peroxyl radicals ($LOO^{\cdot}$) and terminates the lipid peroxidation chain reactions [16]. Carotenoids (Lycopene and β-carotene) are well known to scavenge the peroxyl radicals, disrupting the reaction sequence and preventing the damage to cellular lipids [17]. Lastly, flavonoids, a group of natural benzo-ɣ-pyran derivatives, have a protective effect on the DNA damage induced by the hydroxyl radicals, probably due to the involvement of the chelating metal ions, such as copper or iron, that complexed with the flavonoids prevent the generation of the ROS [18]. A high daily intake of these bioactive components can be easily achieved adding fruits & vegetables concentrate to the daily diet. Indeed, some clinical trials evidenced that this concentrate mix formula (juice or capsules) reduced the markers of oxidative stress, including plasma carbonyl groups on protein (CP) and malondialdehyde concentrations in 41 trained male cyclists and in 48 aerobically trained men after controlled exercise periods (6 capsules/day for 28 and 2 weeks, respectively) [19]. Moreover, a regular fruits & vegetables concentrate intake (4 capsules/day for 11 weeks) in 59 untrained men significantly improved some markers of immune function and oxidative stress, increasing T cells and plasma oxygen radical absorbance capacity while decreasing the plasma cytokine, IFN-ɣ [20]. Asgary and

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Citation: Vadalà M, Palmieri B, Laurino C (2016) Energy Restoration by an Original Fruits & Vegetables Juice Intake in a Cohort of Elderly People Affected by Sarcopenia. Int J Sports Exerc Med 2:046

Received: July 08, 2016: **Accepted:** November 17, 2016: **Published:** November 21, 2016

Table 1: The sarcopenia diagnosis requires documentation of criterion 1 plus documentation of either criterion 2 or criterion 3:

Diagnosis of sarcopenia
1° criterion: Low muscle mass
2° criterion: Low muscle strength
3° criterion: Low physical performance

Table 2: Mechanism that may be involved in the onset and progression of sarcopenia.

Age-related (primary mechanism)	Sex hormones, apoptosis, mitochondrial dysfunction
Endocrine dysfunction	Corticosteroids, GH, IGF-1, abnormal thyroid function, insulin resistance
Neuro-degenerative diseases	Motor neuron loss
Inadequate nutrition/malabsorption	cachexia
Disuse	Immobility, physical inactivity zero gravity

Table 3: Stages of sarcopenia syndrome, according to the European Working Group on Sarcopenia in Older People (EWGSOP).

Stage	Muscle mass	Muscle strength	Performance
Pre-sarcopenia	↓		
Sarcopenia	↓	↓	
Severe Sarcopenia	↓	↓	↓

Downward arrows indicate the criteria for the definition of the stages of sarcopenia syndrome, according to the European Working Group on Sarcopenia in Older People (EWGSOP). The 'presarcopenia' stage is characterised by low muscle mass without impact on muscle strength or physical performance. The 'sarcopenia' stage is characterised by low muscle mass, plus low muscle strength. 'Severe sarcopenia' is the stage identified when all three criteria of the definition are met (low muscle mass, low muscle strength and low physical performance).

coworkers enhanced the antioxidant effect of the orange juice (by high volume intake: 500 mL/twice a day for 4 weeks) in 22 healthy volunteers (18-59 yrs), probably by the combined synergy of two flavonoids (hesperidin and naringenin) that may decrease diastolic blood pressure and might represent a potentially safe energy source [21].

The work hypothesis of our study started by the anecdotal finding of potential energetic benefit of fruits & vegetables juice, supposedly able to interact with the striated muscle mass, thus reducing some expression of sarcopenia (the term sarcopenia describes an age-associated loss of voluntary muscles volume with subsequent measurable reduced performance), a very common finding in the elderly (old people) (Table 1) [22]. This multifactorial disorder encloses age-related mesenchymal and neurological changes, reduced secretion of trophic hormones [23], decrease in muscle innervation and capillary density [24], smoking toxicity [25], extremely sedentary life style, and a quali-quantitative inadequate nutrition [26]; especially a low protein intake or protein metabolism alteration (Table 2 and Table 3) [27]. As to the epidemiology, 25% of people over 70 and 40% of over 80-years-old are found sarcopenic [28].

To date, pharmacological therapies showed limited efficacy in the treatment of sarcopenia, but a multi-ingredient nutrition strategy may offer additional therapeutic potential by targeting multiple factors associated with the development and consequences of sarcopenia, including both physical and cognitive parameters [29].

Indeed several studies defined the benefits between single nutrient intake such as proteins [1-1.2 g/Kg (body weight)/day] [30-34], vitamins, including 800-1000 IU/day of Vitamin D [35], minerals, including Calcium (500 mg/day) [36], and antioxidants (e.g. 160 mg daily of Vitamin E) [37], and reduction of sarcopenia. However, the link between diets and sarcopenia is far from being definitely defined.

The objective of present study has been to assess the energetic effect of fruits & vegetables juice intake in a homogeneous cohort of patients with sarcopenia over an extended period of time (three months).

Materials and Methods

The anecdotal and retrospective observational study recruited an a

Table 4: Baseline characteristics of patients.

Number of patients	30
Mean age (years)	73.5 yrs
Mean height (cm)	165 cm
Mean weight (kg)	68 Kg
Mean BMI (Kg/m^2)	26 Kg/m^2

Table 5: Ingredients of fruit & vegetable juice.

Fruit concentrate	Vegetable concentrate	Other ingredients
Orange juice	Carrot	**Mare's milk**
Lemon juice	Celery	Acerola extract
Passion fruit	Cucumber	Inactive yeast powder
White grape	Sauerkraut	Borage seed oil
Lemon	Beetroot	Rose hip oil
Pomegranate	Onion juice	Rosemary extract
Cranberry	Pepper puree	Aloe vera juice
Chokeberry	Tomato puree	Blossom honey
Strawberry	Broccoli powder	**Emulsifiers (soy lecithin)**
Pineapple	Spinach powder	Nettle extract
Sea buck-thorn	Artichoke leaf extract	Mate tea extract
Redcurrant	Jerusalem artichoke juice	Royal jelly
Elderberry		Garcinia cambogia extract
Blueberry		Birch leaf extract
Blackcurrant		Juniper berry extract
Melon juice granulate		Ginseng root extract
		Curcuma powder
		Dandelion extract

homogenous cohort of 30 patients (27 males and 3 females, age 70-85 years) referred to our "Second Opinion Medical Network" (Modena, Italy) with sarcopenia and weakness during daily activity (Table 1). The "Second Opinion Medical Network" is a consultation referral web and Medical Office System involving a wide panel of specialists to which patients affected by different diseases not adequately satisfied in terms of diagnosis and treatment can apply for a clinical update [38]. Most of the patients, nowadays in fact, often wander around the medical Web-sites, looking for proper answers to their health problems, but this screening is often excessive, compulsive, and sometimes obsessive, leading to the "Web Babel Syndrome (this term expresses a doctor-patient communication gap that especially when affected by multiple synchronous pathologies, feeds back heterogeneous and misleading informations and prescriptions with the risk of a confusionary state [39,40]). To solve this problem, the "Second Opinion Network" represents a useful decision-support tool not only in order to achieve a re-evaluation of the patient's case with a consequent optimization of treatment and prognosis [41], but also to avoid un-necessary investigational procedures, undue unhelpful and expensive medical and surgical treatments [42].

The selected patients were informed, via individual interview, and informed consent previously approved by the Local Institutional Review Board under the Helsinki Declaration. Contemporarily, background information regarding dietary habit (coffee, tea, and alcohol consumption), smoking, and disease history was recorded for each participant.

The specific inclusion criteria were: 1) no smoke, 2) three balanced meals a day (40% proteins, 35% carbohydrates and 25% fats with average 1800 calories/day, to maintain the body weight), 3) no reported food allergies or any food restrictions, namely for the milk proteins and soy lecithin, 4) no drug or nutraceutical admitted affecting appetite or food intake, 5) a body mass index (BMI) within the normal limits (between 20 and 30 Kg/m^2), 6) no diabetes. The following exclusion criteria were: 1) pregnant or lactating women, 2) subjects eating more than average levels of fruit and vegetables (more of 800 grm per day).

The patients were prescribed to drink 20 mL/day (10 mL after lunch and 10 mL after dinner in 120 mL of water/dose) of "Vita Vitale plus" fruits & vegetables juice (Medicura Naturprodukte AG, Burglauer, Germany), composed by fruits concentrate (including

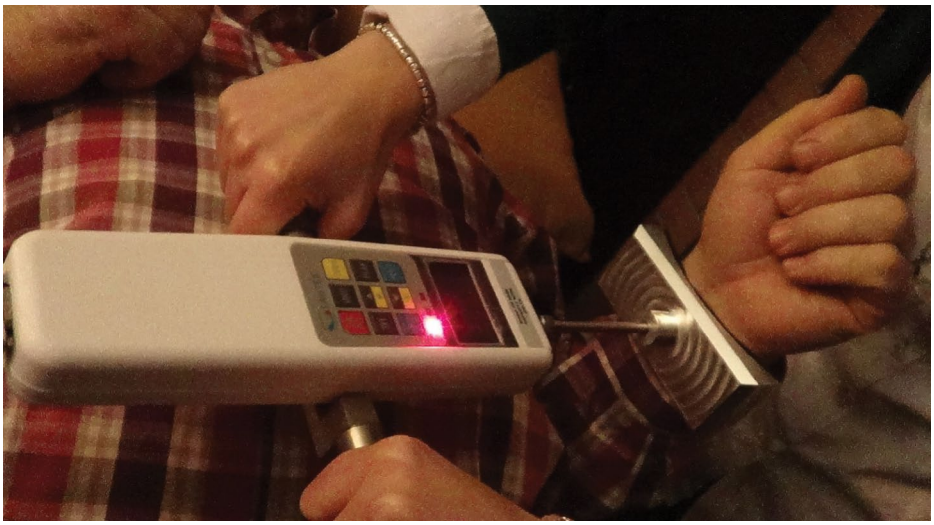

Figure 1: Left elbow extension.

Table 6: Micronutrient composition of the fruit & vegetable juice (per 20 ml). Energy for 20 mL of juice is 150 kj/35 kcal.

Nutrient	Composition	RDA%
Carbohydrates	5 g (sugar 3.8 g)	
Proteins	0.5 g	
Fats	0.6 g (saturated fatty acids < 0.04 g)	
Fibres	< 0.2 g	
Salt	< 0.02 g	
Bitin	104 µg	208
Folic acid	90 µg	44
Niacin	12.8 mg	80
Pantothenic acid	3.7 mg	62
Vitamin B1	1.4 mg	127
Vitamin B2	2 mg	143
Vitamin B6	1.6 mg	114
Vitamin B12	2.4 µg	96
Vitamin C	240 mg	300
Vitamin E	3.2 mg	26.7
Zinc	6 mg	60
Magnesium	56.8 mg	15
OPC	77.4 mg	
Royal jelly	75.2 mg	
L-carnitine	300 mg	
Coenzyme Q10	6.8 mg	

lemon, orange, passion fruit, white grape, pomegranate, cranberry), vegetables concentrate (including carrot, celery, broccoli), mare's milk, and aromatic plants extracts (Table 4, Table 5 and Table 6) for the treatment of three months (two bottles/month). We established the daily intake recommended by the German Company.

Body weight and BMI were recorded before supplementation, on the day-30, day-60 and at the end of treatment (day-90), using a digital instrument for the weight measurement (Kg/m^2).

All subjects had to fill a 20 items questionnaire, divided into physical and psychological fatigue-related symptoms (fatigue, short-term memory, insomnia, depression), gastrointestinal symptoms, possible side effects (nausea, abdominal and stomach pain, vomiting, skin rashes); for which the subjects scored on a 5-point scale (0 for "not at all", 1 for "a little bit", 2 for "somewhat", 3 for "quite a bit", 4 for "a great deal", 5 for "a very great deal"), before and at the end of treatment.

The fatigability severity (energy level) is evaluated (at 1-day and 90-day average) using a standardized athletic performance assessment, such as a 10-minute walk, based on the change a subject perceived in their self-reported energy level over time at the point they stopped walking (i.e. estimate of distance walked, walking speed). Subjects were asked to walk at their self-selected pace for up to 10 minutes. Prior to the walking assessment, each participant was asked to rate their current level of tiredness (e.g. "How tired are you right now?") on a seven-point scale from 7 (extremely tired) to 1 (extremely energetic).

The maximum isometric muscular strength was quantitatively measured by a digital dynamometer "FH-500" (Rupac Srl, Milan, Italy) before and after juice supplementation. Each patient was required to squeeze the muscles as much as possible performing a maximal voluntary isometric contraction for 6 seconds followed by limb relaxation [43]. We carried out four measurements for each patient (elbow extension, hip abduction, knee extension, and ankle dorsiflexion strength) that were repeated three times with intervals of 1 minute, on both right and left sides, and the highest value, expressed in Newton (N), was recorded**.** All the measurements were performed by the same observer and the means of three readings were reported (Figure 1 and Figure 2).

To evaluate the antioxidant effect of fruits & vegetables juice intake in these patients, we used an easy and quick Point of Care Test: Saliva Antioxidant Test (SAT) of FRAS 4 Evolvo (H&D, Parma, Italy). In this test, the sample is prepared chewing for 1 min a cotton square weighing 300 mg and then squeezing the saliva into a small plastic container: 40 µL of R2 reagent (iron solution) is added to the microcuvette containing R1 reagent (thiocyanate derivate pre-dosed solution), followed by 10 µL of the saliva sample. Results are scored by a photometer v505 nm, following a 1-minute incubation period at 37 °C. The principle of SAT test is based on the capacity of an iron solution [solution of ferric ions (Fe^{3+}) bound to a specific chromogen] to discolour when the Fe^{3+} ions are reduced to ferrous ions (Fe^{2+}) in the presence of phosphates [44]. The reduced ferrous ions are thus quantified, expressing the reducing antioxidant status of the saliva in µmol/L of Vitamin C (reference standard used as ferrous-reducing agent). The normal SAT values range are between 1001-1500 µmol/L Vit C, values < 1000 µmol/L Vit C indicate severe shortage of antioxidant status (AS).

Statistical Analysis

The statistical analysis was evaluated using Mann-Whitney test (continuous variables not normally distributed) and chi squared test (categorical variables). A commonly-used measure of linear correlation, the Pearson correlation coefficient, denoted by r, was reported. Statistical significance was set at *p*-value less than 0.05, and all data and graphics were analyzed using the R software, version 3.1.2 [R Core Team, Austria (2015)] [45].

Results

All parametric data, body weight and BMI, showed no differences between the day-1 and day-90 of the treatment. Significant variations ($p < 0.02$) were found in all the patients as to the clinical items before, during and one month after the treatment. Indeed, the fruits & vegetables

Figure 2: Right ankle dorsiflexion.

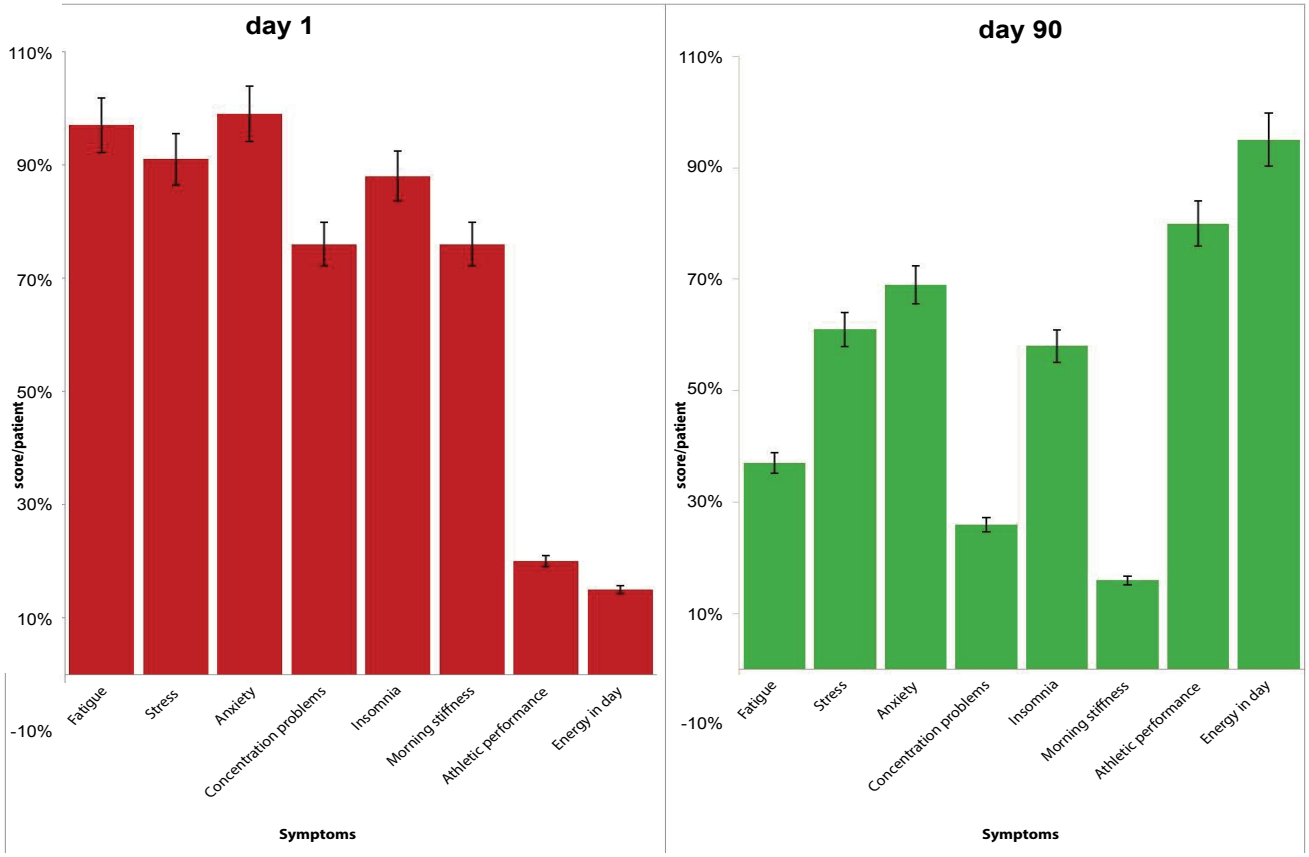

Figure 3: Graphic illustration of the physical and psychological symptoms of the patients at 1-day (before treatment) and at 90th day (after treatment).

juice intake at day-90 significantly reduced stress, morning stiffness and cognitive problems (e.g. concentration problems) and increased the energy level of a each patient previously complaining asthenia and improved sleep quality: 80% of the treated individuals declared less fatigue and more intense athletic performance and 92% expended more energy during the day, measured with a standardized performance assessment, e.g. in walk meters during daily duties (Figure 3).

The four isometric strength values (on both right and left sides for each patient) were significantly increased in all the patients at the end of the treatment compared to measurements performed before juice supplementation (Table 7, $p < 0.001$).

The saliva antioxidant status of the patients, evaluated by SAT Test, significantly increased ($p < 0.03$) at day-90, suggesting that fruits & vegetables juice are well absorbed and can counteract the oxidative stress induced sarcopenia.

Table 7: Values (in Newton) of muscle strength of patients, reported as mean ± standard deviation.

	Value (N) before juice treatment	Value (N) after juice treatment
Left elbow extension	127.2 ± 56.4	185.5 ± 70.5
Right elbow extension	137.5 ± 81.2	161.8 ± 95.3
Left hip abduction	141.6 ± 64.5	180.2 ± 79.0
Right hip abduction	178.2 ± 78.1	192.5 ± 85.0
Left knee extension	171.3 ± 53.1	195.6 ± 54.0
Right knee extension	162.2 ± 48.5	181.3 ± 56.2
Left ankle dorsiflexion	165.05 ± 76.8	193.2 ± 83.2
Right ankle dorsiflexion	166.48 ± 75.4	221.60 ± 83.1

Discussion

The World Health Organization (WHO) recommends to people 400 g/day fruit and vegetables intake [46] and Denmark advices a minimum of 600 g or 6 servings per day [47]. North America, proposes 5-13 servings of fruits and vegetables each day (rated to the caloric intake) [48], and Canada 8-10 servings for adult males under age 50 yrs. [49]. These variable recommendations show that a minimum daily consumption of 5 or more servings of fruits and vegetables is a public health gold standard advice. Kawashima and coworkers, in a randomized double-blind and placebo-trial, showed that supplementation with fruits & vegetables concentrate over a 28-day period in 60 healthy volunteers, produced significant increases in serum antioxidants, such as α-tocopherol (39, 5%), β-carotene (52, 8%) and lycopene (80, 2%) and significant decrease of plasma homocysteine ($p < 0.03$) compared to the placebo group at the end of the treatment [50]. Similar findings were confirmed by Samman, et al. [51] who showed in 32 healthy men that mixed fruits & vegetables concentrate administration (4 capsules/day for 6 weeks) decreased plasma lipid concentrations ($p < 0.05$) by displacing cholesterol-raising constituents of the diet, particularly saturated fat.

Over 250 epidemiological studies, published by the World Cancer Research Fund & American Institute for Cancer Research, suggested the cancer preventive role of fruits & vegetables, due probably to the cumulative role of chemo preventive phytochemicals such as glucosin [52].

Further clinical studies confirmed that consumption of cruciferous and allium vegetables or citrus fruits are linked to antioxidant and antiproliferative effects against specific cancers, including pancreatic, stomach, lung, prostate carcinoma [53-58]. Potential mechanisms for cancer prevention of phytochemicals include prevention of DNA adduct formation [59], enhanced carcinogen elimination [53], inhibition of inflammatory processes [60], interference with tumor angiogenesis [61,62], and a direct cytotoxic effect on tumor cells [63], by folates, polyphenols, anthocyanins, carotenoids, lutein, and lycopene [64-66].

In a further study, a strong reduction of bladder cancer risk was observed in an cohort of population with incident bladder cancer risk (252 men) followed up for ten years period (1986-1996) and consumed (≥ 5 servings/week) cruciferous vegetables, including broccoli, cabbage, Brussels sprouts [58].

A wide variety of phytochemicals but also water-insoluble molecules (including lycopene found in tomato puree and β-carotene found in yellow, orange, and green leafy fruits and carrot, spinach, and broccoli powder) are the basic components of our natural juice, with a potential cancer preventive role actually outside of the scope of the study.

We limited the investigation to the energetic effect by specific clinical questionnaire and dynamometer data.

The prescribed fruits & vegetables juice intake displayed a significant benefit by the antioxidant micronutrients contents, including alpha lipoic acid (present in spinach and broccoli powder), Vitamin E (present in green leafy vegetables), curcumin (present in curcuma powder), supposing that this intake can optimize mitochondrial energy stores, and protect synaptic membranes from lipid peroxidation.

The polyphenols (such as the tannic acid from tea extract) are reported as "sticky" agents binding to the surfaces of the oral cavity (including tongue, palate, gingival epithelium, the teeth and the microbial flora) and neutralizing the oxidative stress [67,68] from the very first tract lumen contact before absorption; this has been clearly shown by the measured salivary antioxidant levels (SAT test).

Conclusions

High dietary intake of antioxidants, especially if joined with healthy lifestyle and physical exercise, can improve the life quality in sarcopenic old patients, reducing asthenia, improving energy level and sleep. Our protocol has been proven effective to relieve the symptoms usually requiring a complex chronically administered drug regimen.

In the tested juice, we find a well balanced tailored integration of vitamins and natural phytocomplexes, with the specific claim to fulfill the ancient aphorism: "The food will be your primary drug" in the prominent concept to take care of an healthy oral intake, namely with a regular vitamin level, which is often reduced especially in elderly people frequently complaining of malabsorption and food restriction.

Our easy & safe diet integration is low-moderate-cost effective treatment that can be used in combination with usual drug therapies of the old ages, with no reported side effects.

Competing Interest

The authors declare that they have no competing interests.

Acknowledgements

The authors thank the Company Medicura Naturprodukte AG (Burglauer, Germany) for the fruits & vegetable juice.

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