Design management of functional foods for quality of life improvement

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Abstract

The paper examines the benefit of bread enriched with antioxidants on oxidative stress, and on the quantities of hydrosoluble antioxidants in a group of human subjects. The home–management of functional foods strategy seeks to improve prompt and effective basic nutrition using additional attributes that are directly positively beneficial for health and well–being. The purpose of this clinical study was to test the tolerance and benefits of multicomponent functional foods enriched with antioxidant compounds obtained from plant extracts on healthy adult volunteers. A detailed protocol was created to formalize and standardize the procedures for data collection, e.g. filling out standardized forms and functional diet questionnaires. For the research method, Group A was given the special diet enriched with multicomponent antioxidant foods and Group B (control). The data were analysed using the quantitative methods. They showed significant increase of hydrosoluble antioxidants in group A compared to control, from 220.61+/–27.92 – 313.56+/–37.09 micrograms/mL (p=0.05), compared to 280.47+/–32.1 – 238.27+/–44.93 micrograms/mL (p=0.45). Also, oxidative stress values showed a decrease in the diet group compared to control that reached statistical significance. Oxidative stress decreased in the diet group to 244 +/- 89 compared to 308+/-108 UFORT in the control group. The responses of the prevention of chronic diseases to a functional foods strategy depend on how they are absorbed and utilized in the body. An anti–oxidant diet with natural bioactive components could become an interesting solution for degenerative disorders in which oxidative stress is increased.

Key words

multicomponent foods, health, hydrosoluble antioxidants, oxidative stress

INTRODUCTION

The reason for these indications is to make both people with disease and health care providers aware of beneficial nutrition interventions. This involves the use of ideal available scientific tests while taking into account treatment arguments, strategies to attain such cases, and individuals with diseases (including cardiovascular diseases). The foods are primarily accepted for their essential nutrients for normal body activity and function. A functional food is analogue in countenance to, or may be a conventional food, is consumed as part of a normal diet, and established as having physiological benefits and/or reducing the risk of chronic disease beyond basic nutritional functions. One of life's paradoxes is the fact that the molecule supporting aerobic life – oxygen – is not just essential for energetic metabolism and respiration, but almost equally involved in the ethiopathogenesis of numerous diseases and degenerative states, due to oxygen–based reactive species called free radicals [1, 2]. Free radicals (FR) occur in certain oxido–reduction reactions in which structural modifications take place, resulting most often in a change in the biological function of the substance (it becomes more hydro–soluble or/and becomes involved in another chain of metabolic reactions) [3]. Nature has picked and included in an evolutionary manner in the structure of living bodies, reactions generating FR with multiple roles: functional, inter–cellular communicational or destructive, cytolytic, etc. If, at molecular level, the main target of the FR is the sulphhydril (–SH) free or protein groups, at cellular level the main target are the cellular membranes. FR occurs in the body as the result of endogenous metabolic function, or of the local assimilation of some noxious at cellular level or at the level of several tissues, simultaneously or progressively.

Due to their superior reactivity, FR have been found responsible for many noxious benefits on the living body [3, 4, 5]. The connection between food and health has long been known to exist. Currently, the basic concept of food is changing from one that involves the conservation of life to one that uses food to improve health and contribute to a better quality of life [6]. As a result, there is great interest in this relationship by public health officials, consumers, and the food sector industry. These foods, referred to as 'health foods', were originally described as 'functional foods' and the food industry is reflecting this change in the production and marketing of functional foods [7, 8]. Antioxidant is any substance that reduces oxidative damage caused by FR which ultimately results into chronic diseases [9]. Dietary behaviour is one of the most significant factors that have impact on chronic disease. The formation of reactive oxygen species (ROS) is the result of the incomplete diminution of molecular oxygen during cellular metabolism. Although ROS have been shown to act as signalling molecules, it is known that these reactive molecules can act as pro–oxidants causing damage to DNA, proteins and lipids, which over time can lead to disease propagation and ultimately cell death [10].

Thus, restoring the protective antioxidant capacity of the cell has become a significant target in therapeutic intervention. The generation of ROS occurs under normal physiological conditions that cause damage to biological structures. To counteract the pro–oxidant load, a diversity of anti-oxidant defense systems are operative in biological systems, including...
enzymatic and non-enzymatic anti-oxidants [11]. The human diet contains an array of compounds that possess antioxidant activities or have been suggested to scavenge ROS based on their structural properties. The body’s antioxidant defence system is capable of being alerted by dietary means [12]. A strategy for balancing the oxidative damage and antioxidant defence of human cells and tissues would be to enhance the antioxidant capacity by optimizing the dietary intake of antioxidants.

Another approach may be to neutralize oxidative compounds in the diet [13], and the content and bioavailability of antioxidants. Bioavailability of antioxidants depends on several food-related and host related–factors [14]. A well-recognized food-related factor is the amount of antioxidant in a meal; for nutrients which are absorbed by a process of passive diffusion, the proportion of antioxidant absorbed decreases with increasing amounts in the food [15, 16]. The molecular forms of antioxidants in foods, e.g. in which isomers or molecular linkages such as the esters exist [17, 18], are also significant determinants of bioavailability. Furthermore, the food matrix in which antioxidants are located often influences availability of the nutrient [19]. The potential implications related to antioxidant nutritional enhancement include all degenerative diseases, since oxidative stress plays a major role in the pathways that lead from health to disease. The substances to be regarded are the anti-oxidant nutrients vitamin C and E, as well as non-nutrient anti-oxidants, mainly flavonoids and carotenoids, which may be significant in the overall anti-oxidant protection afforded by the diet [20, 21]. From the point of view of safety, the benefit of anti-oxidants must strictly be concerned with their benefits in vivo; however, the possibility that a given compound may be converted by chemical reaction, or bacterial action, into a toxic substance within the gastro-intestinal tract, or during storage of the food that contains it, must also borne in the mind because such products may themselves be absorbed and exert their toxicity in vivo [22, 23, 24, 25]. ROS play a role in the pathogenesis of many diseases including cardiovascular diseases. Several methods have been developed for the direct or indirect measurement of the oxygen free radical and its by-products [26, 27, 28].

The current study was designed to validate the free oxygen radicals test and to investigate the potential relationships between the distinctive diet enriched with multicomponent antioxidant foods, and clinical or biological factors in patients with roles in the prevention of chronic diseases. This study aims to provide insights for food producers into the territory of health-claimed foods. The data was compiled from research studies that analyzed in depth the induction of consumer acceptance of functional foods. This study recognizes, exposes and reviews the sources of data for multicomponent functional foods. It is designed to support, extend and track public health nutrition policies and services. Attention to diet-health relationships has passed to the forefront of public health concerns in several areas of the developed world.

MATERIALS AND METHOD

A prospective experimental study design was adopted. The study lasted for a period of 3 months in 2008, with a follow–up at the middle of the period and at the end of the 3rd month. At inclusion, follow-up and end of the study, the following metabolic parameters were measured and compared in both groups: blood lipids, glucose, uric acid, oxidative stress, hydro– and liposoluble antioxidants. Group A filled out a sensory and food tolerance questionnaire regarding functional meals. There were no restrictions on drink, except for alcohol and sweet soft drinks, which were forbidden during the study. Consumption of more than one cup of coffee, green or black tea was also forbidden, as they could alter the results. Smoking was refrained during the whole study.

Clinical research protocol. A prospective experimental study of the parallel design type was adopted. The purpose of this clinical study was to test the tolerance and benefits of multicomponent functional foods, enriched with antioxidant compounds obtained from plant extracts, on healthy adult volunteers. The criteria for inclusion were males or females aged between 22–25 years; BMI < 25 kg/m2; without any medication and with a pliable diet. The subjects were randomly allocated to the intervention group or to the control group in identical numbers. The baseline diet for both groups was designed for a caloric intake of 35 kcal/kg/day opportune to moderate physical activity. All diets were constant for fat or fibre. There were 7 menu days. The intervention group A received a functional meal daily with a fixed quantity of multicomponent functional foods represented by 150 g of enriched bread with 0.5% nutraceutic (capsaicin extracted from red peppers, magnesium from barley germ and minerals), 300 g raw vegetable salad with olive oil and balsamic vinegar from apples and honey and 0.5 % buckthorn extract rich in beta-carotene, followed by 2 tablespoons of the by-product (30 mL), an alcoholic extract of grape seeds and skins. At the same meal, group B served as a comparison group and received 150 g white flour bread with 300 g raw vegetable salad with olive oil and ordinary wine vinegar. These meals were prepared under the surveillance of the investigators in the hospital kitchen, and remained identical. For the other meals of the day, all subjects involved in the study kept daily diaries. The daily intake of meat allowed during the study was 150g/day (raw weight), chicken, fish or beef.

Quantitative methods. Blood glucose was determined by the colorimetric method glucooxidase–peroxidase kit (Clinical Lab. Diagnosticum, Budapest, Hungary). For serum total cholesterol the enzymatic colorimetric method was used (Clinical Lab. Diagnosticum kit). Serum triglycerides were determined by the enzymatic colorimetric method, also using the above kit. HDL–cholesterol was determined by an Vitalab–Flex–PC analyzer. LDL was calculated using the Friedewald method, LDL=TC–HDL–TG/5. The level of circulating insulin was determined by the ELISA method; kit DSI–10–1600 ACTIVIE Insulin Enzyme–Linked Immunoabsorbant (ELISA) (DRG Diagnostics GmbH, Marburg, Germany). The HOMA Insulin Resistance Index (IRHOMA) was calculated according to the known formula: \( \text{Fasting Insulin (U/mL)} \times \text{Fasting Glucose (mmol/L)} / 22.5 \). Hydrosoluble and liposoluble antioxidants were determined using standards of vitamin C and of trolox, by photochemoluminometry (Photochem Analytik Jena, Germany).

Oxidative stress was expressed by total radicalic activity of whole blood determined by the FORMox free oxygen.
radicals test, a colorimetric method based on Fenton’s reaction, approved for clinical studies (Bethesda), normal values < 310 UFORT [17, 29, 30]. Before the start of the study, 20 volunteers were given, in random order, 50 g of carbohydrate portions of the enriched bread with nutraceutical 0.5%. Venous blood samples were taken when fasting and then 30, 60, 90, 120, 150 and 180 min after the start of the meal. The results of glycaemia were plotted against the results for each volunteer after intake of 50 g glucose in order to determine the glycaemic index of the bread. Palatability and sensory analysis was recorded using a questionnaire developed by the Food Hygiene Department.

**Ethical considerations.** The study was approved by the local Ethics Committee. The volunteer subjects gave their consent to participate in the study, in accordance with the ethical principles of bio–medicine for studies on human subjects. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national), and performed in accordance with the ethical principles of the Helsinki Declaration (World Medical Association Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects) revised in 2000, and which are consistent with applicable government regulations and institutional research policies and procedures, and applicable regulatory requirements and in conformance with the applicable International Conference on Harmonization (ICH) Guidelines on Good Clinical Practice (CGP). The electronic data recording data system used for this clinical research study has not been fully certified, due to the limited scope of this clinical research study.

**Confidentiality.** Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act.

**Statistical analysis.** Performed using STATA 9/Student’s t test, p values <0.05 being considered statistically significant.

**RESULTS**

The study was conducted on 2 groups of healthy volunteers (n = 20 in each group), aged 21–25 years (mean age = 22.81), M = 8, F = 12. Group A (functional diet) was given the special diet enriched with the multicomponent antioxidant foods described above. Group B served as the control group. The characteristics of the 2 groups at inclusion compared to the values obtained after completion of the study are given in Tables 1 and 2. The normal value of oxidative stress was considered below 310 Fort units and of hydrosoluble antioxidants above 80 micrograms/mL. Table 1 shows the characteristics of group 1. There were 20 young adult volunteers, 4 males and 16 females. The blood glucose, cholesterol, HDL (high-density lipoprotein), LDL (low-density lipoprotein), and triglycerides (TG), magnesium, gamma glycercyl transferase, uric acid, oxidative stress and hydrosoluble antioxidant values were within normal limits, except in 3 individuals with increased total cholesterol, 6 individuals with increased oxidative stress values, and 2 individuals with decreased hydrosoluble antioxidant values. Table 2 shows the characteristics of group B.

### Table 1. Characteristics of group A (n=20) at study start and completion of study.

<table>
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<tbody>
<tr>
<td>1</td>
<td>Glicemia (mg/dl)</td>
<td>20</td>
<td>78.87</td>
<td>4.93</td>
<td>72.1</td>
<td>90.2</td>
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<td>2</td>
<td>CT (mg/dl)</td>
<td>20</td>
<td>175.76</td>
<td>22.27</td>
<td>132.2</td>
<td>211.3</td>
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<tr>
<td>3</td>
<td>TG (mg/dl)</td>
<td>20</td>
<td>80.83</td>
<td>38.31</td>
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<td>158.9</td>
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<td>4</td>
<td>HD (mg/dl)</td>
<td>20</td>
<td>54.93</td>
<td>8.96</td>
<td>39.8</td>
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<td>LDL (mg/dl)</td>
<td>20</td>
<td>104.99</td>
<td>28.59</td>
<td>61.4</td>
<td>161.4</td>
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<td>6</td>
<td>Mg (µg/dl)</td>
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<td>0.446</td>
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<td>1.97</td>
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<td>7</td>
<td>GGT (U/L)</td>
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<td>13.68</td>
<td>5.88</td>
<td>1.78</td>
<td>27.7</td>
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<tr>
<td>8</td>
<td>Acid_oric (µg/mL)</td>
<td>20</td>
<td>4.20</td>
<td>1.34</td>
<td>2</td>
<td>6.6</td>
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<tr>
<td>9</td>
<td>Form_ox_U (µg/mL)</td>
<td>20</td>
<td>308.66</td>
<td>108.32</td>
<td>160</td>
<td>600</td>
</tr>
</tbody>
</table>

Data are presented as mean+SD.

### Table 2. Characteristics of group B (n=20) at study start and completion of study.

<table>
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<tbody>
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<td>1</td>
<td>Glicemia (mg/dl)</td>
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<td>2</td>
<td>CT (mg/dl)</td>
<td>20</td>
<td>166.65</td>
<td>21.78</td>
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<td>3</td>
<td>TG (mg/dl)</td>
<td>20</td>
<td>66.27</td>
<td>30.71</td>
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<td>HDL (mg/dl)</td>
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<td>5</td>
<td>LDL (mg/dl)</td>
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<td>95.56</td>
<td>18.27</td>
<td>62.68</td>
<td>122.88</td>
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<td>6</td>
<td>Mg (µg/dl)</td>
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<tr>
<td>7</td>
<td>GGT (U/L)</td>
<td>20</td>
<td>13.12</td>
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<td>20.5</td>
</tr>
<tr>
<td>8</td>
<td>Acid_oric (µg/mL)</td>
<td>20</td>
<td>3.74</td>
<td>1.12</td>
<td>2.1</td>
<td>5.6</td>
</tr>
<tr>
<td>9</td>
<td>Form_ox_U (µg/mL)</td>
<td>20</td>
<td>244.25</td>
<td>89.46</td>
<td>148</td>
<td>512</td>
</tr>
</tbody>
</table>

Data are presented as mean+SD.
There were 20 young adult volunteers, 4 males and 16 females. The blood glucose, cholesterol; HDL, LDL triglycerides, magnesium, gamma glyceryl transferase, uric acid, oxidative stress and hydrosoluble antioxidant values were within normal limits, except in 6 individuals with increased total cholesterol, 4 individuals with increased oxidative stress values and 2 individuals with decreased hydrosoluble antioxidant values. The values for oxidative stress and hydrosoluble antioxidants were compared between the 2 groups, and between study start and month 3 in each group by using the two-sample t test with equal variances. The results are given below and show a significant increase of hydrosoluble antioxidants in group A, compared to control, from 220.61 +/- 27.92 micrograms/mL to 313.56 +/- 37.09 micrograms/mL (p = 0.05), compared to 280.47 +/- 32.1 micrograms/mL to 238.27 +/- 44.93 micrograms/mL (p = 0.45) (Tab. 3). Also, oxidative stress values showed a decrease in the diet group compared to control that reached statistical significance. Oxidative stress decreased in the diet group to 244 +/- 89 UFORT (Fort units) compared to 308 +/- 108 UFORT in the control group.

### Table 3. Comparison of hydrosoluble anti-oxidants in group A and group B after 3 months of antioxidant diet.

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Study start</th>
<th>Month 3:</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Diet group</td>
<td>220.61 +/- 27.92</td>
<td>313.56 +/- 37.09</td>
<td>p = 0.05</td>
</tr>
<tr>
<td>B Control group</td>
<td>280.47 +/- 32.1</td>
<td>238.27 +/- 44.93</td>
<td>p = 0.45</td>
</tr>
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</table>

Data are presented as mean +/-SD.

### DISCUSSION

Observational epidemiologic studies have shown that an ample consumption of consumed multicompontent diet (fruits and vegetables) is associated with a decreased risk of chronic diseases and age-related degenerative disorders. In the presented study, successive to the diet enriched with multicompontent antioxidant foods, total hydrosoluble antioxidants in serum determined by photochemoluminometry significantly increased from 220.61 +/- 27.92 microg/L to 313.56 +/- 37.09 in group A (p = 0.05), compared to control. This increase was associated with a significant decrease in oxidative stress, from 308.66 +/- 108.32 UFORT to 244 +/- 89.46 UFORT (p = 0.05), retaining normal range in all subjects and proving the antioxidant benefit of this multicompontent diet. The RDA (recommended daily intake), which is not established for carotenoids and polyphenols, is defined to prevent nutrient deficiencies and does not take into account the diminution of risk of chronic diseases. The quantities of antioxidant nutrients effective in the diminution of risk of chronic diseases generally lie higher than the RDA. Considering that antioxidants have significant roles in the prevention of chronic diseases, the question is: what would be the best interval of intake recommended? Poor amounts of antioxidants have been observed in smokers, the elderly, and in patients with typical diseases or risk factors, and several studies have demonstrated that the intake of antioxidants may be suboptimal in certain peoples.

However, in other locations where the option of products is restricted or more seasonal, a number of groups of people are not able to meet the stringent requirements for vitamins E, C or beta-carotene. At peculiar risk are the less affluent and the elderly. A new review of current literature denotes that fruits and vegetables in combination have synergistic benefits on antioxidant activities leading to further diminution of risk of chronic disease, specifically for cancer and heart disease [31, 32]. In the presented study, this synergistic antioxidant benefit seems to have been achieved by the combination within the foods tested of the most significant classes of antioxidants – carotenoids and polyphenols, a benefit proved in group A by the significant decrease of FORMox and by the increase of integral antioxidant capacity of hydrophilic substances in serum (ACW).

Epidemiological studies are necessary to quantify the impact of antioxidants on disease etiology. Intervention trials formally test the efficacy of enhanced intake of antioxidants. In evaluating these health benefits, preferably hard endpoints (disease incidence, recurrence and mortality) should be used. There are a number of groups of functional foods that offer a diversity of benefits. The following Table lists some of them along with the source of the food and the capacity health benefits [30].

A promising approach to strengthen epidemiological studies is the use of biomarkers. A biomarker can be defined as an indicator on a biochemical, genetic or cellular level, and reacting on exposure to stress, susceptibility for a disease, or the health status of a subject. A biomarker of oxidative stress refuses radical burden, oxidative damage, and oxidative stress averaged disease, or health status. Other intermediate end-points may be effective, provided that they are genuinely predictors of the disease of interest. In work on functional foods, the development and application of biomarkers of exposure (dietary intake), biomarkers of biological response and of subclinical disease, and biomarkers of susceptibility, should be used.

For anti-oxidants, all kinds of markers have evident significance. For instance, blood quantities of vitamin E (an exposure marker) may be studied in relation to oxidation resistance of LDL–C (a biological response marker) or to carotid artery wall thickness (a disease marker), in subjects with familial hypercholesterolaemia, or typical genotype (both susceptibility markers). If biomarkers have the capacity for improving validity and reducing bias, several issues are encountered [33, 34]. Biomarkers of exposure should exactly reflect the relevant dietary intake or body status, and early disease markers should have predictive value for the hard end-point. As chronic diseases have vast latency periods, requiring large initial numbers to appreciate health benefits, biomarkers of intermediate end-points may, in certain conditions, should lawfully be used more efficiently [4, 35, 36]. Lots of dietary antioxidants may contribute to cellular protection against radicals and other ROS. Additionally, dietary antioxidants include vitamins and carotenoids. Vitamin E (8 structural isomers of tocopherols/tocotrienols) is one of the most widely distributed antioxidants in nature [27, 37], and it is the primary chain-breaking antioxidant in cell membranes. Among these, α-tocopherol is the best known and possesses the most antioxidant activity. In addition to its direct antioxidant properties, growing evidence suggests that some of the beneficial benefits of vitamin E in cells reside in its ability to regulate gene expression of proteins.
An effective nutritional strategy will require knowledge of the type of antioxidants in the diet, their sources, bioavailability and required quantities of intake for protective benefits [38, 39]. The bioavailability of antioxidants from raw fruits and vegetables is generally low, but heat treatment of the food and concomitant intake of fat increases their bioavailability. At the same time, heat treatment may lead to loss of antioxidants and isomerisation, i.e. formation of cis-isomers. From dietary supplements, antioxidants bioavailability is higher for compounds not associated with the plant matrix, and fat in the form of vegetable oil is part of the formulation. Protective profits of antioxidants have been found in mechanistic studies in vitro and in vivo, and epidemiological studies and certain intervention studies have provided useful information. However, it is appropriate to consider the totality of the evidence from basic science, epidemiology and intervention studies, rather than to rely on the evidence from any one type of study. Currently, the recommendation is to increase the intake of a consumed multicomponent diet (mix of fruits, vegetables and functional foods). Aerobic organisms utilize oxidative catabolism as a highly effective method of extracting energy from food molecules. The data presented in the present study has been adjusted for confounders. Thus, values are different from those obtained for the same parameter in the descriptive statistical analysis. The results appreciated with the linear growth statistical model, are described with two parameters: a baseline value and a rate of increase for an investigated parameter. The level of uric acid was significantly higher after the intervention study but stayed below the risk level of 7.0 mg/dL – 5.7 mg/dL for males and females, respectively. Also, the clinical parameters for uric acid, total cholesterol, LDL-cholesterol, HDL-cholesterol and tryglicerides, showed a statistically significant change. Interesting differences regarding the responses to the intervention were obtained when each individual was observed separately. An antioxidant diet with natural bioactive components could become an interesting solution for degenerative disorders in which oxidative stress is increased. Incorporation of natural active components with therapeutic profits in functional foods, based on results of multidisciplinary research, will be a challenge for both the food and drug industries. The FORM system provides this field with a simple method for oxidative stress screening, as well as monitoring the benefits of any lifestyle changes and antioxidant therapy.

Limitations. the present study has some limitations for the people in various parts of the. world because some have different views on the nature of functional foods. Since high antioxidant quantities in foods do not necessarily translate into quantities found in the body, and the capacity health benefits of these antioxidants ultimately depend on how they are absorbed and utilized in the body, the presented study was undertaken to determine antioxidant diet with natural bioactive components.

CONCLUSIONS

This study has demonstrated the diapason of factors that influence the checking-up of functional foods strategy aims, and their subsequent realization revealed key tests that can support searching for such diets. Many areas could greatly benefit from the introduction of functional foods, although relatively little is known about the current status of production and market development in most of these areas. There is a need to assess functional foods and its increase in capacity because such data is needed to make investment decisions; the data is also needed to support the design and implementation of development and research aimed at straightening the value added features of agricultural and food processing activities. The increase in vitamin and micronutrient quantities can be explained in two ways. Firstly, the adherence of the volunteers to the programme: compliance with taking portions of the diet each day should lead to higher quantities of micronutrients and vitamins. Secondly, the selection of typical food items consumed in the multicomponent diet, high in exemplary antioxidants/ vitamins, explains the higher quantities of an exemplary micronutrient. Epidemiologically it has been shown that a multicomponent diet, rich in fruit and vegetables, lowers the risk for degenerative diseases. The advantages in exposure to a multicomponent diet rich in those foods is confirmed by the quantities of carotenoids and vitamins measured in blood. In addition, the multicomponent diet should be beneficial for markers of biological response.

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