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# Analysis of health-related biomarkers between vegetarians and non-vegetarians: A multi-biomarker approach

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ABSTRACT

This study was performed in a group of adult vegetarians (N = 40) and matched non-vegetarian subjects (N = 40) in order to analyse differences in health-related biomarkers. Obtained results revealed differences in various biomarkers between subjects on a traditional mixed and vegetarian diet, indicating that vegetarians have a lower nutritional status of some nutrients (Ca, Cu and Zn, and vitamins $B_{12}$ and D) accompanied with a lower antioxidant defence system (glutathione) and higher homocysteine and genome damage (micronuclei and DNA strand breaks), along with shorter telomeres. This suggests that the supplementation of animal derived nutrients to this particular dietary group would be beneficial for the improvement of some measured health-related biomarkers. However, the level of certain toxic metals (As and Hg) was higher in non-vegetarians. The presented multi-biomarker approach implies the necessity of evaluating a large number of different health-related biomarkers in order to obtain clear insight into dietary preferences and health outcomes.

## 1. Introduction

Diet is one of the key environmental factors affecting the incidence of various chronic health disorders and there is growing evidence that a vegetarian diet as well as specific components of a vegetarian diet promote health and longevity and lower the incidence of many chronic diseases including cancer. The different vegetarian diets show the variability in the extent to which animal products are avoided which can then influence micronutrient intake (Kazimírová et al., 2006; Krajcovicová-Kudlácková, Valachovicová, Pauková, & Dusinská, 2008; Majchrzak et al., 2006). Vegetarian diets are classified according to whether they contain no animal products (vegan) or if they include dairy products (lacto (L)-vegetarian), eggs (ovo (O)-vegetarian) or both (lacto-ovo (LO)-vegetarian) (Gaby, 2013). A vegetarian diet may result in a higher intake of some vitamins and micronutrients, which provide antioxidant defence, but it may also lead to a deficiency in others involved in DNA metabolism and stability, such as B group vitamins (Kazimírová et al., 2006).

Antioxidants from food have an important role in cellular antioxidant defences. Antioxidant substances in a diet enhance the DNA, protein and lipid protection by modulating several signalling pathways and gene expression, protecting and repairing DNA damage and increasing the free radical scavenging ability that occurs during metabolic reactions (Khuda-Bukhsh, Das, & Saha, 2014; Nosrati, Bakovic, & Paliyath, 2017; Varoni, Lo Faro, Sharifi-Rad, & Iriti, 2016). It has been shown that a human diet with a high intake of fruits or vegetables rich in antioxidants decreases the level of oxidative DNA damage (Duthie, Ma, Ross, & Collins, 1996; Key, Appleby, & Rosell, 2006; Pool-Zobel,

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Bub, Müller, Wollowski, & Rechkemmer, 1997). Since DNA damage is the initiating event in carcinogenesis, this supports the idea that fruits and vegetables might be beneficial in cancer protection by preventing free radical attacks on macromolecules (Kazimírová et al., 2006; Wasson, McKelvey-Martin, & Downes, 2008). Antioxidants of plant origin such as vitamins C and E, carotenoids and flavonoids are involved in protection from diseases by decreasing the level of oxidative damage (Smolková et al., 2004). Folate is an essential B vitamin that occurs naturally in a wide variety of foods, such as broccoli, cabbage, cauliflower, fruit and nuts. Its synthetic oxidized form, folic acid, is used in fortified foods and vitamin supplements because it is more stable than the natural reduced glutamated form. Since mammals are unable to synthesize folate *de novo*, they are bound to obtain it from dietary sources. Inadequate folate intake is associated with increased risk of neural tube defects, Down's syndrome, cardiovascular disease, Alzheimer's disease and various cancers (Beetstra, Thomas, Salisbury, Turner, & Fenech, 2005). These findings may indicate that vegetarianism increases antioxidant protection, implying a possible reduction in the risk of cardiovascular diseases and cancer (Kazimírová et al., 2004).

On the other hand, meats and dairy products are sources of vitamin $B_{12}$. Vitamin $B_{12}$ is necessary for cell division and erythropoiesis and its deficiency can cause anaemia, lesions of the nervous system and an increased level of homocysteine related to increased risks for cardiovascular disease, Alzheimer's disease and atherosclerosis (Herrmann, Schorr, Purschwitz, Rassoul, & Richter, 2001; Obersby, Chappell, Dunnett, & Tsiami, 2013). Vitamin D, important in calcium metabolism, is present mostly in food of animal origin. However, humans get vitamin D from exposure to sunlight, from their diet, and from dietary supplements. Dietary sources of vitamin D include fatty fish, cod liver oil, egg yolks, portabella mushrooms, beef liver, and fortified foods such as breakfast cereal, milk (dairy and non-dairy), infant formula, cheese, and orange juice (Holick, 2007; Pfotenhauer & Shubrook, 2017). In most vegetarians, iron, calcium and total protein concentrations are lower, which may lead to DNA damage and oxidative stress (Ames, 1999, 2001). Therefore, minimising or eliminating animal products from the diet decreases the intake of some essential nutrients. Hence, the lack of balance between the amount of "unhealthy" and "healthy" food also leads to the accumulation of unrepaired damage, initiating DNA instability and the possibility of inducing cancer development (Kapiszewska, 2006).

Exposure to different chemicals is also an issue that has to be considered when assessing differences in the dietary intake of a certain population. The assessment of the diet should also include various pesticides, herbicides, and other potentially toxic agricultural chemicals, mycotoxins, food additives, heavy metals, environmental contaminants and compounds formed during food processing. Exposure to pesticides and other toxic compounds may be lower in vegetarian diets due to their accumulation in animal tissues (Gaby, 2013). On the other hand, higher intake of fruit, vegetable and cereals may result in higher exposure to pesticide residues and mycotoxins (Leblanc, Tard, Volatier, & Verger, 2005; Van Audenhaege et al., 2009).

Therefore, the aim of our study was to compare the effects of vegetarian *vs.* non-vegetarian diet using a large number of health-related biomarkers including haematological, biochemical and oxidative stress parameters, and to see whether differences in one's diet influence genomic instability and impact telomere length. Essential and toxic elements, pesticides and mycotoxins were also measured in biological samples of both groups as well as bone mineral density (BMD). The results of the present study offer complex insight into the differences of selected biomarkers related to specific dietary preferences, which could directly benefit clinicians and nutritionists in patient counselling regarding nutrition and dietetics.

## 2. Subjects and methods

### 2.1. Participants

The study was performed in a group of 40 healthy adult vegetarians (average age 31.93 ± 7.23, range 19–55 years), 24 women and 16 men, and a group of matched (by age, gender and smoking habit) healthy adult non-vegetarians (average age 31.58 ± 7.67, range 22–59 years). The percentage of active smokers was 17.5% in both groups. There were more women than men in this study, which is expected as there are generally more women who practice a vegetarian diet than men (Phillips, 2005). In the vegetarian group, there were 30 LO-vegetarians and 10 vegans. The average period of vegetarianism was 8.85 ± 4.69 years (range 3–20 years). Semi-vegetarians who consumed small quantities of chicken and fish were excluded from the study. Both vegetarians and non-vegetarians were recruited from the general Croatian population. All of the subjects lived in the same region (Zagreb and surroundings), had similar patterns of physical activity and similar levels of education (high school and university). None of the assessed subjects had been exposed to ionizing radiation or steroid therapy for at least 6 months, or antibiotics for at least 3 months before the study. The weight and height of the volunteers varied between 47 and 116 kg and 155 and 200 cm, respectively with a mean body mass index (BMI) of 22.85 ± 3.04 kg/m$^2$. Height and weight were measured using a portable stadiometer and electronic scale. BMI was calculated as weight (kg) divided by the square of height (m$^2$).

A detailed questionnaire with general (age, gender) and anthropometric data (weight, height) as well as life style (smoking, alcohol), health status and dietary habits was filled in for each subject. The section regarding dietary habits was based on the non-quantitative food frequency questionnaire (FFQ). Population characteristics are given in Table 1 and dietary factors for both groups gathered by the questionnaire in Table 2. The study was approved by the Ethics Committee of the Institute for Medical Research and Occupational Health, Zagreb, Croatia. The subjects were informed about the aim and experimental details of the study and all gave their written informed consent. No private details on the subjects involved in the study have been or will be disclosed in public.

### 2.2. Sampling

Blood was collected by venipuncture from fasting subjects under aseptic conditions between 8 and 9 am. Details regarding the blood sampling collection tubes are given in Supplementary material (Table S1). Blood samples were immediately used for common biochemistry and haematology parameters, as well as cytogenetic assays and oxidative status measurements, while blood, plasma and serum for other tests were stored at −80 °C until analysis. After collection, all of the blood samples were handled in the same manner. They were blindly coded,

**Table 1**
Population characteristics in vegetarians compared to non-vegetarians.

| | Non-vegetarians | | Vegetarians | |
|---|---|---|---|---|
| | mean ± SD | range | mean ± SD | range |
| Gender (M:F) | 16:24 | – | 16:24 | – |
| Age (years) | 31.58 ± 7.67 | 22–59 | 31.93 ± 7.23 | 19–55 |
| Height (cm) | 173 ± 10 | 155–198 | 174 ± 10 | 158–200 |
| Weight (kg) | 70.48 ± 15.82 | 47–116 | 68.65 ± 13.06 | 50–99 |
| BMI (kg/m$^2$) | 23.29 ± 3.38 | 18.11–29.60 | 22.42 ± 2.63 | 18.31–28.04 |
| Smokers (%) | 17.50 | – | 17.50 | – |
| Vegetarianism (years) | – | – | 8.85 ± 4.69 | 3–20 |

M – male; F – female; BMI – body mass index.
Results are presented as means ± SD (standard deviation of the mean) including their ranges.