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Effects of Raw Food Diet on Cardiovascular and Immune System in Post-Menopausal Women and its Possible Benefits in Context of Covid-19 Pandemic: Case Study

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Abstract

COVID-19 pandemic continues to spread all over the world and there are no indications of weakening. Clinical presentation and general outcome of COVID-19 are mainly dependent on cardiovascular and immune status of COVID-19 patient prior to infection. It is suggested that the consumption of uncooked food could have many health benefits such as improving function of immune system and reducing the risk for cardiovascular diseases but there are no prospective studies supporting such conclusions. Therefore, we wanted to determine exact effects of six months raw food diet on cardiovascular and immune system as well as on main haemato-biochemical parameters as possible prevention of worse Covid-19 outcome. This prospective study was performed on two 50-year old, postmenopausal, otherwise healthy females. While negative control continued to consume conventional diet, the subject followed exclusively a raw omnivorous diet during the study. During the study, physical activity of volunteers was unchanged. Status of cardiovascular system was determined by treadmill ergometry and immune status was followed on peripheral lymphocytes by flow cytometry. The most important haemato-biochemical parameters were monitored monthly. We found that six months long raw food diet increases frequency of innate immune cells, especially NK cells. It also improves heart rate and systolic pressure recovery after treadmill ergometer exercise although biochemical blood analysis showed increased levels of total cholesterol and LDL and decrease in iron levels. Our results indicate that raw food diet improves cardiovascular and immune system and therefore could provide protection from worse Covid-19 outcomes.

Keywords: *Raw Food Diet; Immune System; Cardiovascular System; Cholesterol; Covid-19*

Introduction

COVID-19 is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that leads to significant modifications of patient immune system resulting in increased levels of proinflammatory cytokines and decreased number of adaptive immune cells (CD3+ T cells, CD4+ T cells, CD8+ T cells, and B cells) and NK cells [1-4]. Clinical presentations can vary, and obesity and hypertension are associated with severe cases [5]. Most of laboratory findings in COVID-19 patients include increased CRP, LDH, ALT and

AST levels [6] and significantly decreased levels of HDL, LDL and cholesterol [7]. Raw food diet (rawism) is associated with strengthening of the immune system [8,9], with lower risk of hypertension, obesity, diabetes and cancer [10-12], with reduction or alleviation of the symptoms of rheumatoid arthritis [13] and with prolonged lifespan in general [9, 11, 14]. The basic principle of raw food diet (rawism) implies consumption of uncooked food or food heated below 46°C, but the type of food consumed can vary [15]. There are three types of raw food diet: (a) a raw vegan diet that excludes all animal products and only plant-based food can be consumed; (b) a raw vegetarian diet that includes plant-based foods plus raw eggs and unpasteurized, non-homogenized dairy products; (c) a raw omnivorous diet that includes plant-based foods and all raw animal products, including meat [16]. Namely, most of research related to rawism are focused on possible health impact of substances that are altered or produced during thermal food processing. Food enzymes are destroyed above 40°C - 49°C and at the same time harmful toxins, such as trans-fatty acids (TFA) and advanced glycoxidation end products (AGE) are formed [12,17,18]. TFAs are thought to increase the risk of cardiovascular disease due to positive linear correlation between TFA intake and elevated blood LDL cholesterol associated with decreased concentrations of HDL cholesterol [19]. Studies have also shown that increased TFA intake is correlated with type-2 diabetes [20], postmenopausal breast cancer [11] and colon cancer [16]. AGEs, pathological structures in which sugars and amino acids are tightly bound into a non-degradable compound, inhibit physiological function of different molecules and cause irreversible damage to vital organs [18]. Furthermore, raw foods contain bacteria and other microorganisms that have a beneficial effect on the immune system and are destroyed by heating [8]. Despite the fact that there are numerous positive effects of rawism on human health, it is important to point out some negative aspects, including lower bone density [15], low HDL cholesterol levels and vitamin B12 deficiency [21], amenorrhea, and malnutrition in women [22] and increased risk of food poisoning [23].

Considering all possible benefits of raw food diet, the aim of this study was to investigate the direct effect of six months long raw food diet on cardiovascular and immune system as well as on some biochemical markers and to discuss the findings in context of Covid-19 disease.

Material And Methods

Study design and subjects

The study had prospective design, lasted six months and was performed on two 50-year old, postmenopausal, otherwise healthy females. Prior to the study volunteers fallowed a conventional diet, mostly cooked foods, and raw foods in the form of salads or fruits. One volunteer (negative control) continued to consume conventional diet during the whole study, while the second followed exclusively (100%) a raw omnivorous diet (subject further in the text) for the next six months. The first 15 days were a transitional period during which subject's diet consisted of approximately 80% raw food and 20% cooked food. The daily menu consisted of five meals, breakfast, lunch, dinner and two snacks (Table 1).

Meal	Food type
Breakfast	Green juice pulp about 1L, (e.g. one handful of chard leaves + apple + banana)
Snacks 1	Fruit or handful of nuts (almonds, walnuts, cashews or mix)
Lunch	Raw fish or meat with unprocessed vegetables
Snacks 2	Fresh or dried fruit (dates, raisins, plums)
Dinner	Yogurt or kefir from raw milk

Table 1: *Example of the daily menu.*

During the study, physical activity of volunteers did not change. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Clinical Hospital Center of Rijeka (Project Number: uniri-pr-biomed-19 - 171498). All volunteers provided written and informed consent prior to participation in the study.

Methods

Ergometry

Ergometry was performed according to the Bruce protocol [24] at the beginning and at the end of the study in order to determine effect of rawism on cardiac performance. Briefly, Bruce test has 7 levels, each lasting three minutes. The initial slope (10%) and speed (2.73 km/h) progressively increase up to 22% and 9.6 km/h. According to weight, height and age, the maximal predicted heart rate is calculated. Measurable parameters are: pulse, blood pressure and electrocardiogram. The test score is time taken on the test (in minutes) or achieved MET level (metabolic unit).

Haemato-biochemical parameters

Haematological parameters, erythrocytes, thrombocytes, leukocytes, haemoglobin and differential blood count were determined monthly by Advia 212i automatic analyzer.

Biochemical parameters, blood sugar, urea, creatinine, bilirubin, alfa-amylase, GGT, AST, ALT, cholesterol, HDL, LDL, triglycerides, Fe, UIBC, TIBC, Na, K and Cl were determined monthly by COBAS C 6000 automatic analyzer.

Flow cytometry

Peripheral blood cell immunophenotyping was performed at the beginning and at the end of the study. Freshly isolated lymphocytes (3×10^5 cells/sample) from subject and negative control were labelled for surface CD3/CD56/CD16 or CD19/CD5 or CD3/CD4 or CD8 antigen detection and analyzed by flow cytometry (Becton Dickinson FACSCalibur) using CellQuestPro software (BD Biosciences, San Jose, California, CA). Detail protocol can be seen in Link., *et al.* [8].

Results

Raw food diet improves cardiovascular system

Status of the volunteers' cardiovascular system was determined by ergometry at the beginning and at the end of the study. Pulse, systolic and diastolic blood pressures were monitored. Ergometry testing was stopped after 9 minutes i.e. at the 10.2 MET. The results after six months of raw food diet shown lower heart rate at the 10.2 MET (154/min vs. 172/min) and shorter recovery time (1 min vs. 5 minutes) (Figure 1.A).

Trend of pulse (A) and systolic and diastolic blood pressure (B) were monitored during exercise and during recovery period according to the Bruce protocol.

Diastolic pressure had less oscillation during the load test although resting and recovery values did not change. Resting and recovery values of systolic pressure were lower while at maximum load the values were higher (Figure 1.A). At the same time ergometric findings of the negative control did not show remarkable changes (data not shown).

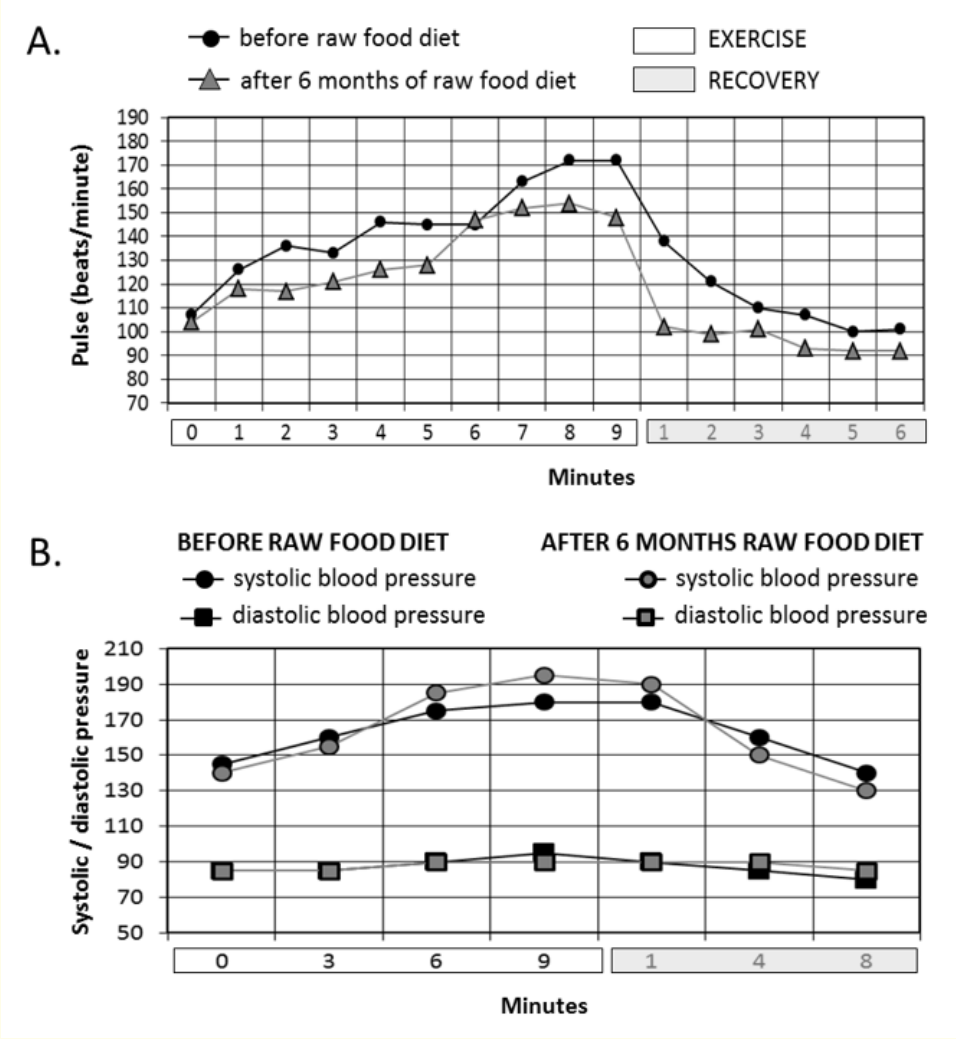

Figure 1: *Results of ergometry before and after six months of raw food diet.*

Immune status changed after raw food diet

The frequency of the lymphocyte subpopulation in freshly isolated mononuclear suspension from peripheral blood were analysed by flow cytometry within R1 gate at the beginning and at the end of teh study (Figure 2). Among investigated lymphocyte subpopulation, only the frequency of $CD56^{+}$ cells increased after six months of row food diet (1.7 fold change) while the frequency of other investigated immune cell populations remained mainly unchanged (Figure 2). At the same time flow cytometry analysis of peripheral blood lymphocytes of the negative control did not show notable changes (data not shown).

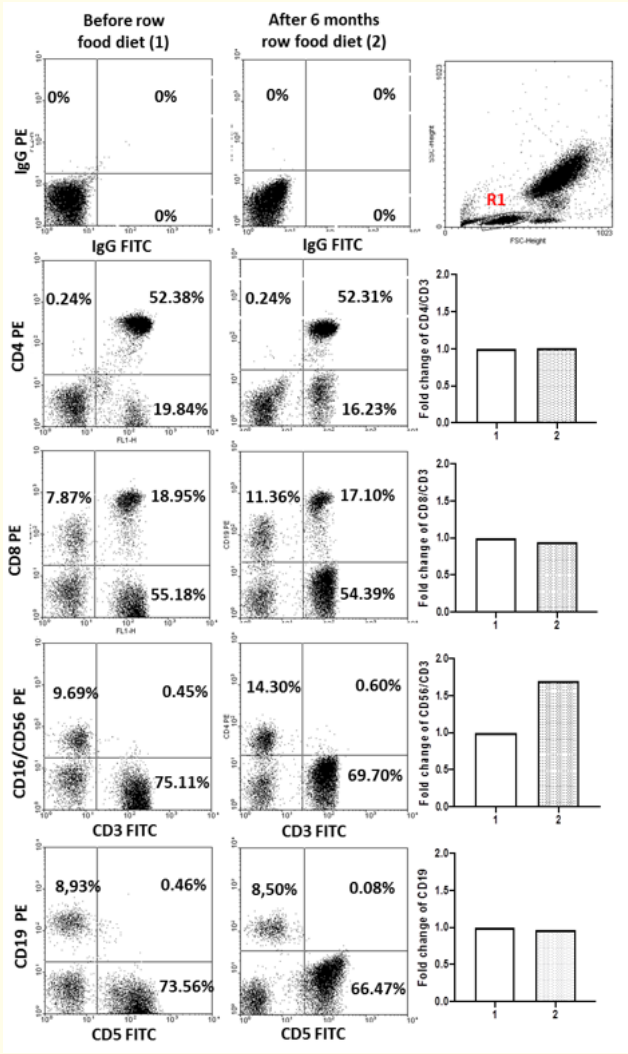

Figure 2: *Flow cytometry analysis of lymphocyte population (R1 gate) from peripheral blood before raw food diet (left column) and after six months of raw food diet (middle column).*

The result of the lymphocyte subtypes analysis is shown as the percentage of positive cells to the corresponding differentiation marker. Graphs in right column represent the fold change of different lymphocyte subtypes before and after raw food diet.

Evaluation of haemato-biochemical parameters during six months raw food diet

Volunteers' haematological parameters (erythrocytes, thrombocytes, leukocytes, haemoglobin and differential blood count) and biochemical parameters (blood sugar, urea, creatinine, bilirubin, alfa-amylase, GGT, AST, ALT, cholesterol, HDL, LDL, triglycerides, Fe, UIBC, TIBC, Na^+, K+ and Cl^-) were determined monthly. All investigated parameters of the negative control shown only slightly variations (data not shown). In contrast, after six months of raw food diet subject´s iron decreased (from 20 to 13 µmol/L) and UIBC increased (from 41 to 49 µmol/L) (Table 2).

Parameter	Duration of raw food diet in months					
	0	1	2	3	4	6
Iron (µmol/L)	20	14	12	14	15	13
UIBC (µmol/L)	41	42	49	47	44	49
Cholesterol (mmol/L)	5,8	5,0	5,2	6,4	6,3	6,6
LDL (mmol/L)	3,9	3,4	2,3	2,9	4,4	4,7
HDL (mmol/L)	1,5	1,4	1,3	1,6	1,5	1,5

Table 2: *Hemato-biochemical parameters.*

Although subject´s cholesterol and LDL levels decreased at the end of second month (from 5,8 to 5,2 mmol/L and from 3,9 to 2,3 mmol/L), at the end of the study we found increased levels of cholesterol and LDL (6,6 and 4,7 mmol/L, respectively). HDL levels did not change (Table 2). All other subject´s investigated parameters shown only slightly variations (data not shown).

Discussion

Although the volunteers' physical activity was unchanged during the study, the subject´s final ergometry has shown decreased resting pulse values, decreased pulse values at maximal load as well as shorter recovery period after six months of raw food diet. Resting and recovery values of systolic pressure were lower while at maximum load the values were higher (Figure 1). This data imply that raw food diet could improve heart contractility, possible due to lower uptake of harmful food additives (hormones, antibiotics etc.) and starch. Namely, starch induces production of carbonic acids that dissociates to H^+ ions that competitive bind to troponin C and decrease heart contractility [25]. Lower resting blood pressure values and better heart contractility could improve Covid-19 infection outcome because higher blood pressure is associated with higher mortality [5,26]. Initially, raw food diet caused decrease of cholesterol and LDL concentrations but at the end of study those concentrations were much higher. This increase of cholesterol and LDL concentrations is probably not direct result of raw food diet but rather consequence of weight loss since the subject lost 7 kg between second and sixth month of raw food diet. Namely, due to increased beta-oxidation to meet the energy needs there is increased synthesis of endogenous cholesterol in the liver that finally results in elevated blood concentrations of cholesterol and LDL [27]. Considering that higher body weight is poor prognostic factor, body weight normalisation could result with better outcome of Covid-19 disease [5,26].

Immunophenotypisation showed an increase of NK cells frequency after six month rawism, while other lymphocyte subpopulations did not show notable changes. Those results indicate strengthening of nonspecific immunity, important for anti-viral and anti-tumoral protection. It is possible that unprocessed food can boost and stimulate proliferation of NK cells due to increased uptake of vitamins and polyphenolics in fresh fruit and vegetables, and n-3 fatty acids in fish [9]. The similar mechanism could be the cause of increased ratio of CD4:CD8 (2.7 to 3.1) and switch from cellular to humoral immunity which that suppresses inflammation, one on the most important hallmarks of cancer [8]. Negative control did not show any variations of immune status during six months period.

During the raw food diet, trend of iron decrease and UIBC increase was observed probably due to a lack of red meat intake from which iron is more easily resorbed than from plant foods due to the bivalent (ferro) form [28].

This study is very significant considering that it is the only study performed in controlled conditions showing cardiovascular and immune status as well as haemato-biochemical parameters before and after six months of raw food diet in the same person. Furthermore, results of this research raise a possibility that raw food diet could be protective against worse outcome of Covid-19 infection due to lower resting blood pressure values, better heart contractility, and weight normalization together with stimulation of innate immunity. Rawism

practitioners must be careful with weight loss due to possible increase of cholesterol and LDL and iron levels have to be monitored. Further studies that will include higher number of participants are needed in order to confirm those results.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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Conceptualization: GBZ, EC and TG; investigation: EC and GBZ; data curation: TG and GBZ; writing: GBZ and TG; supervision GBZ.

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