A human study to evaluate toxic trace element detoxification, body fat reduction following four-week intake of the Wellnessup diet: a three-arm, randomized clinical trial

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Su-Jin Jung
Chonbuk National University Hospital

Woo-Lim Kim
Chonbuk National University Hospital

Byung-Hyun Park
Chonbuk National University Medical School

Seung-Ok Lee
Chonbuk National University Hospital

soowan chae
Chonbuk National University Hospital

Corresponding Author
swchae@jbctc.org
ORCiD: https://orcid.org/0000-0003-3660-8272

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Abstract
Background: Detox diet are known as a popular dieting strategies that helps toxins elimination and weight manage but there is very little clinical evidence. Methods: To evaluate the effects of 4 week intake of the Wellnessup diet (WD) on toxic trace element detoxification, body fat reduction, and safety parameters. Forty-five women with body mass index (BMI) of 23.5-30 kg/m² were recruited. Thirty of them were assigned 1:1 to the test group (WD, 15 subjects) and control group 1 (calorie-restricted diet, CRD, 15 subjects) in a single blind and randomized, and the remaining 15 subjects were assigned to control group 2 (maintaining regular diet, MRD). The primary outcome were toxic trace element levels in hair (29 types of heavy metals), and the secondary outcomes were changes in anthropometric and urinary organic acids. Results: The levels of four toxic trace elements in hair decreased in the WD group after the diet compared to before the diet. Ni, Rh, Sn, and Ga were significantly lower in the WD group than in the CRD or MRD group (p<0.05). A significant weight loss in both groups (WRD:-1.88±0.95kg; p<0.013, CRD:-3.22±0.48kg; p<0.01). The BMI, fat free mass and hip circumference (HC) of the CRD group were not only significantly decreased compared to the WD group but also Weight, BMI. Body fat mass, fat free mass, waist circumference, and HC were significantly decreased (p<0.05) compared to the MRD group, and the weight loss was a greater decrease was observed in the CRD group. No significant changes in any safety parameter were observed. Conclusions: Use of WD might have several beneficial effects and safety such as body fat reduction and improving some the element detoxification through caloric restriction but did not reducing body fat mass more than calorie-restricted diet.

Introduction
Recently, with increasing industrialization, different chemicals and heavy metals have polluted the air and water, contaminating food and affecting health. Most of the environmental pollutants delivered through food are heavy metals and persistent organic pollutants (POPs). Chronic exposure to and accumulation of these pollutants have been linked to non-communicable diseases (NCDs) such as obesity, diabetes, cancer, cardiovascular diseases, and chronic respiratory disease [1–4]. Recent reports have indicated that accumulation of Toxic trace elements in the body is related not only to
metabolic disorders that cause overweight or obesity, but also to greater energy intake in people with obesity and diabetes. Furthermore, the diets of obese individuals often result in an imbalance of trace nutrients, and sufficient intake of vitamins and minerals has been emphasized in the management of obesity [5]. Detox dieting has become a popular strategy to promote toxin removal and weight loss, thus enhancing health and improving quality of life. However, detox diets are controversial, as some argue that there is a lack of scientific evidence for their health benefits, or that such diets may even be harmful [6–9]. Nevertheless, food-based nutrients have been studied for their ability to modulate metabolic pathways involved in detoxification processes. Several preliminary studies have demonstrated that food extracts and nutrients can regulate the transduction and eventual excretion of toxins [10–15]. Typically, detox diets are calorie-restricted diets consisting of a single fruits, vegetables, or beverages (tea, vinegar, lemon juice, salt water, or drinks mixed with micronutrients) [10–25]. The diet detox program known as the lemon diet and hypocaloric Mediterranean diet [16, 26] were a very low-calorie diet (LCD) that allows 500 to 1,000 kcal per day and is effective in reducing body weight and fat; however, this dietary intervention is difficult to maintain and can lead to shortages of minerals, vitamins, and dietary fiber, as well as increased binge eating and stress [27, 28]. Moreover, while fasting or LCDs may allow weight loss, they can also lead to various health problems, such as malnutrition, muscle weakness, nervousness, headaches, dizziness, fatigue, gastrointestinal disturbances, and reduced quality of life [29, 30]. Several pre-clinical studies have reported the effects of detox diets [31–33], mostly with respect to detoxification, and some clinical studies have been published [10–16, 18–20, 22, 24–26].

In general, the effect of the detox diet on obesity was assessed for the weight loss effect in a short term period, but the biomarker was not measured for the detoxification effect of harmful elements. Furthermore, no scientific studies have investigated the effectiveness of nutritionally balanced detox diets for weight loss and toxin elimination.

The Wellnessup diet (WD) was designed as a healthy meals based on organic plant based diets including various vegetables, fruits, whole grains, nuts and phytonutrients [34]. We performed a four-week, single blind, randomized, controlled pilot study to evaluate the efficacy and safety of the WD
and CRD for toxic trace element detoxification and reduction of body fat in overweight young women.

Materials And Methods

This study was reviewed and approved by the Institutional Review Board (IRB) (CUH 2017-11-009) of Chonbuk National University Hospital (CBNU). The entire human study was conducted in accordance with the provisions of the Helsinki Declaration and the standards for clinical trial management (IGCP), and the protocol was registered at Clinical Research Information Service of Republic of Korea (https://cris.nih.go.kr/cris/en/: board approval number: KCT0003002). This study was performed from January 2018 to March 2018 as a single-organization, randomized, parallel-group-controlled clinical study.

Subjects

The subjects in this study were recruited from the Clinic Trial Center for Functional Foods (CTCF2) at CBNU through advertising (brochures, posters) and the CBNU website. Volunteers were evaluated for eligibility after providing written consent. Suitable subjects for this study were selected through screening tests such as medical interviews, medical examinations, and diagnostic medical examinations within four weeks (Day – 28 to Day – 1) of the baseline evaluation date (Day 0). To meet the criteria for selection, subjects needed to be women older than 19 and younger than 49 years at the time of the screening test, with body mass index (BMI) of 23.5 to 30 kg/m². The subjects received and fully understood the detailed description of this human study, voluntarily decided to participate, and agreed in writing to comply with the precautions.

Subjects who met any of the following criteria were excluded from this study:

1) those who had lost more than 10% of their body weight within three months before the screening test; 2) those who had taken medicines or health supplements related to detoxification or weight loss within one month prior to the screening test; 3) those with clinically significant acute or chronic diseases of the cardio-cerebrovascular system, endocrine system, immune system, respiratory system, hepatobiliary system, kidney and urinary system, nervous system, or musculoskeletal system or with inflammatory diseases or blood tumors; 4) those with a history of gastrointestinal disease (e.g., Crohn's disease) or gastrointestinal surgery (excluding simple appendectomy or herniotomy)
that could affect absorption of the study diet; 5) those with hypersensitivity reactions to the ingredients of the study diet; 6) those who had taken antipsychotic drugs or narcotic analgesics within six months prior to the screening test; 7) those suspected of drug abuse or a history thereof; 8) those drinking alcohol in excess of 21 units/weeks or have a history of alcohol abuse; 9) those with serum AST or ALT level three times greater than the upper limit of the reference range, or serum creatinine level over 2.0 mg/dL in diagnostic examinations; 10) those who had participated in other studies within two months of the screening test; 11) those who were in menopause (for more than 12 months) or perimenopause period (for a continuous period of 3 months or more); 12) those who were pregnant, breastfeeding, or planning to become pregnant during the study; 13) those who did not agree with the use of effective contraception methods (condoms, contraceptives, intrauterine contraceptives, and male partners with vasectomy) during the study period; and 14) those who were deemed unfit for this study by the tester due to diagnostic examination results or other reasons.

Study design
Among 61 volunteers who provided written consent to participate in this study, 45 who met the selection and exclusion criteria were selected. Among the 45 selected subjects, 30 were assigned 1:1 to the test group and control group 1 by a single-blind and random arrangement method, and 15 were assigned to control group 2. A total of 45 participants were randomly assigned into one of the study groups (15 subjects each) using a computer-generated random number table by the Randomization program of the version 9.2 SAS® system (SAS Institute, Cary, NC, USA). The subjects were to consume the respective study diets for four weeks (test group, WD; control group 1, CRD; control group 2, MRD). The average daily energy values of the menus provided to the test group and control group 1 are shown in Table 1. All 45 of the registered subjects completed all the procedures and examinations specified in the test plan.

Dietary interventions
Experimental diet (Wellnessup diet: WD)
The diet of the test group (WD) consisted of organic ingredients produced by smart farms (pesticide-free and pollution-free). The menu consisted of breakfast (shakes), lunch (fresh fruit and vegetable juice), dinner (salads), and snacks (nut bars) and was followed on 14 days cycle. The ingredients in the
shakes included whole-food materials to promote detoxification while supplementing grain, and fruits powder. The shakes were delivered in powder form in individually packed sticks, which were to be opened, poured into a shake bottle, combined with at least 250 mL of water (the amount of water used is a matter of personal preference), and mixed well before consumption. For lunch, 450 mL of vegetable juice (a form made by grinding various raw fruits and vegetables) was refrigerated and shaken before consumption. For dinner, the salad consisted of about 400 g of organic green vegetables, whole grains, meat, and fruit, along with a salad dressing. The nut bars was made using a variety of dried fruits and roasted nuts. Which was recommended as snacks between lunch and dinner and were to be stored at room temperature (Supplementary Table 1).

Control group 1 (Calorie-restricted diet: CRD)
The diet of control group 1 was similar to the WD in its dietary weight and caloric content. This calorie-restricted diet consisted of a shake for breakfast, fruit and vegetable juice for lunch, salad for dinner, and nut bars for snacks, as in the test group. The CRD was composed of general ingredients purchased from supermarkets. Shake powder products for market sale (grain and yogurt) were used for breakfast, and fruit juice products for market sale were served for lunch. Salads consisting of vegetables, fruit, meat, and whole grains cultivated by general farming were served for dinner, and nut bars were provided as snacks. For breakfast, the shake was prepared through addition of at least 250 mL of water (based on personal preference) in a shake bottle, mixed well before consumption. For lunch, 450 mL of refrigerated vegetable juice was shaken and ingested. For dinner, the salad consisted of about 400 g of green vegetables, whole grains, meat, and fruit, along with a salad dressing. The nut bars were recommended as snacks between lunch and dinner and were to be stored at room temperature.

Control group 2 (Maintaining regular diet: MRD)
The subjects assigned to control group 2 were required to maintain their daily meal patterns without calorie restriction for four weeks and did not receive provided meals. The subjects recorded all foods and beverages consumed for 28 days in detail on a diet record form.

Compliance and safety of the study subjects
We recommended that the subjects eat only the clinical research diet provided during the study
period and asked them to maintain the same level of physical activity that they were performing before the study to minimize the impact of lifestyle changes on the test results. To observe changes in diet and physical activity, we assessed the dietary intake, diet compliance, and physical activity levels of the subjects during the study period. For examination of diet, subjects were asked to record the foods they consumed every day in a dietary diary. Subjects were monitored for current drug use, self-reported symptoms or side effects, changes in physical activity, lifestyle habits, and suitability for the chosen diet. A safety test was conducted to evaluate the clinical condition of each subject, including adverse reactions, and the results were recorded in a case report to investigate possible adverse reactions in this human study. In addition, vital signs, blood tests, and chemical tests were reviewed.

**Outcome Measures**

**Anthropometric measures, harmful elements in hair, and biochemical analysis**

All subjects underwent efficacy and safety assessments before and after the four-week study. The primary efficacy evaluation items were harmful elements in hair (29 types of heavy metals), and the secondary efficacy evaluation items were anthropometric indexes (weight, BMI, body fat, body fat percentage, fat-free mass, waist circumference(WC), hip circumference(HC), and waist-to-hip ratio(WHR)), lipid metabolic indexes (total cholesterol, Apo A1, Apo B, triglycerides, HDL-cholesterol, and LDL-cholesterol levels), blood glucose indexes (glucose, insulin, HbA1c), an inflammatory index (hs-CRP), uric acid level, GGT level, and urinary organic acid levels (β-hydroxybutyrate, isocitrate, methylmalonate, α-ketoisocaproate, α-hydroxybutyrate, 3,4-dihydroxyphenylpropionate, and 8-hydroxy-2-deoxyguanosine). As safety measurements, abnormal responses were monitored, a diagnostic examination was performed, vital signs were tested, a physical examination was conducted, and an electrocardiogram was recorded.

**Anthropometry And Medical Examination**

The anthropometric measures were height, weight, WC, HC, and BMI. Height and weight were measured with a GL-150 (G-Tech Co., Uijeongbu, Korea) while subjects were dressed in light clothing. WC was measured with a tape measure around the middle of the pelvis and the lower part of the ribs while the subject stood with his/her feet 25–30 cm apart and breathed comfortably. Body fat, body fat
percentage, and muscle mass were measured with an Inbody 720 (Biospace Co., Seoul, Korea).

Blood pressure measurement

Blood pressure was measured with an HBP-9020 (Omron Healthcare Co., Ltd, Kyoto, Japan) after the subject had arrived at the research site and had rested comfortably for at least 10 min. Three measurements of systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse rate were recorded at intervals of approximately 2 min while the subject was seated, and the average was calculated. The medical team carried out the examination through interviews, ocular inspection, auscultation, percussion, and palpation.

Harmful elements in hair

In total, 29 harmful elements were measured in hair by means of an ICP, Agilent 7800 ICP-MS (Agilent, CA, USA)[35]. Twelve carcinogenic heavy metals (arsenic, beryllium, cadmium, lead, mercury, nickel, palladium, rhodium, thallium, tin, thorium, and uranium) and 17 toxic heavy metals (aluminum, antimony, barium, bismuth, cerium, cesium, gadolinium, gallium, gold, indium, platinum, rubidium, silver, tellurium, titanium, tungsten, and zirconium) were identified in hair. For collection, 0.4 g of hair was cut as close to the roots as possible from 5–6 places in the occipital region. Hair was also collected at the nearest point to the scalp (two to three cm from the scalp), and long hairs or long hair ends were not used. It was recommended that the subjects wash their hair with only water on the day of hair sampling (sweaty or dirty hair was not suitable).

Blood test

Blood was collected after subjects had fasted for more than 12 h overnight. The blood was centrifuged at 3,000 rpm (Hanil Science Industrial Co., Ltd. Seoul, Korea) for 20 min and kept frozen at −80 ºC until analysis. Total cholesterol, neutral blood lipid, and HDL-C levels were analyzed with a Hitachi 7600 – 100 analyzer (Hitachi High Technologies Corporation, Tokyo, Japan), and the LDL-C content was calculated with the Friedewald formula [36]. Glucose, insulin and HbA1c levels were measured to investigate blood glucose control. Lipid metabolic indexes of free fatty acid, apolipoprotein A1, apolipoprotein B, and apolipoprotein E, along with liver enzyme indexes of GGT, ALT, AST and total bilirubin, were analyzed with an ADVIA 2400 chemistry system (SIEMENS, Munich, Germany).
Urinary organic acid test
The intermediate urine of the first urine of the morning (about 10 mL) was collected from each subject, and urinary organic acid tests were conducted. The urine samples were analyzed for levels of β-hydroxybutyrate, isocitrate, α-ketoisocaproate, methylmalonate, and α-hydroxybutyrate (AHB) by GC-MS (Agilent 5977B Series GCC/MSD System, Santa Clara, CA, USA). The 8-hydroxy-2-deoxyguanosine (8-OHdG) level was analyzed by LC-MS (Agilent 5977B Series GCC/MSD System). The water intake of the subjects was limited to one cup (240 mL) after 8 p.m. the day before urine sampling. The subjects were also instructed to refrain from excessive concentrate, drinks, coffee, and alcohol.

Investigation of dietary intake and physical activity
Trained nutritionists explained the dietary diary to each subject and provided instructions for use. The dietary intake surveys were collected at the first (baseline) and second visits, and the results were verified directly through interviews when the data were retrieved. The subjects completed the first dietary intake survey three days prior to the first visit in the dietary diary according to the meal record method. The purpose of the first survey was to determine the total calorie and nutrient composition of the average daily diet before the study. The second dietary intake survey was conducted to determine the intake of the study diet and all the other foods (beverages) for 28 days. The subjects assigned to the MRD group recorded all the foods they ate freely every day in the diary. The randomly assigned subjects in the WD and CRD groups marked whether or not they had eaten the study diet each day in the issued diary. The subjects in these groups also recorded their intake levels of the provided foods and photographed the remaining amounts after they had eaten the meals each day. Other foods and beverages consumed were also recorded in detail on a separate form. Even if the subjects ate meals other than those provided in this study, they were not eliminated. The subjects were taught to accurately record the additional foods that they would inevitably consume, after receiving training to eat only the foods that were provided and those that were added to the served foods. In the analysis of dietary intake data, the average values of the dietary diaries for 28 days were evaluated by the researchers assigned to each group in the Can Pro 4.0 program (The
Korean Nutrition Society, Seoul, Korea). Physical activity was evaluated according to a metabolic equivalent task (MET) assessment using the global physical activity questionnaire (GPAQ). The MET value was used for analysis of physical activity or GPAQ data, representing the relative proportion of working metabolic rate to metabolic rate at rest.

Statistical analysis
All statistical analyses were performed using SAS® version 9.2 (SAS Institute, Cary, North Carolina, USA). The mean ± standard deviation (SD) was used for continuous variables, and the frequency was determined for categorical variables. The Chi-square test and Wilcoxon rank-sum test were used for the homogeneity test and the baseline homogeneity test, respectively, among the study groups. The variation in primary and secondary outcomes efficacy evaluation item after four weeks of diet intake (Δ Week 4 - Week 0) was compared among the WD, CRD, and MRD groups by the Kruskal-Wallis test and the Repeated Measures ANOVA. Control groups 1 and 2 were compared with the test group by the Wilcoxon rank-sum test. For each group (WD, CRD, and MRD group), the changes in intake before and after the four-week study were evaluated by the Wilcoxon signed-rank test. The significance was statistically significant at the level of p < 0.05.

Sample Size
The sample size was statistically calculation for this study was based on the assumption of -2.6 kg variation in measured weight after 7 days of Lemon detox diet supplementation, -0.6 kg variation in the control group, and standard deviation of 1.7 kg, a sample size of each group was determined to be 15 participants, allowing for a 20% dropout rate. The groups were equal in size in order to obtain the greatest statistical power. The number of subjects required was calculated as described in Kim et al. (2015) [16] therefore, a total of 45 participants were randomly assigned into one of the study groups.

Results
Participant demographic characteristics
The general characteristics of the subjects in this study are presented in Table 2. All the study subjects were women, and the average age was 26.2 ± 4.6 years. The age, weight, BMI, blood pressure, pulse, blood glucose and lipid profiles, drinking history, and smoking history did not differ
Significantly among the three groups. Sixty-one subjects voluntarily provided written consent and were screened for this study, and 45 of them were selected following evaluation of suitability for the study. No one was allowed to drop out or violate the research plan during this study, and the 45 registered subjects completed all the study procedures (Fig. 1).

Changes in harmful elements in hair
The changes in toxic trace elements in hair are presented in Table 3. A total of 29 heavy metals, three were significantly lower or exhibited a reduction after four weeks of WD intake compared to baseline (Ni, p = 0.003; Rh, p = 0.005; W, p = 0.002). Among these four heavy metals were significantly lower in the WD group compared to the CRD or MRD group (Ni, p = 0.025; Rh, p = 0.034; Sn, p = 0.050; Ga, p = 0.042).

Changes in anthropometric indexes
The results of the anthropometric indexes are presented in Table 4. After four weeks of diet intake, the WD group exhibited significant decreases in weight (p = 0.013), BMI (p = 0.011), fat-free mass (p = 0.002), and WC (p = 0.0067) compared to baseline. In the WD group compared to the CED or MRD group weight (p = 0.007), body mass index (p = 0.001), and WC (p = 0.007) were significantly lower after four weeks of participation in the diet. Reduction change of fat-free mass was also significantly lower in the WD group than in the CRD or MRD group (p = 0.002). Although fat-free mass was reduced in both the WD group (p = 0.046) and the CRD group (p = 0.019), a greater decrease was observed in the CRD group.

Changes in urinary organic acids
The results for the urinary organic acids are presented in (Supplementary Table 2).

The levels of isocitrate (p = 0.041) and α-ketoisocaproate (p < 0.001) in the WD group were significantly increased after the diet than at baseline. In the CRD group, the levels of β-hydroxybutyrate (p = 0.003), methylmalonate (p = 0.002), and 8-hydroxy-2-deoxyguanosine (p = 0.030) were significantly different after four weeks of the diet. Significant changes in methylmalonate (p = 0.041) and 8-hydroxy-2-deoxyguanosine levels (p < 0.001) were observed in the MRD group after four weeks. The change in methylmalonate level differed significantly between the WD and MRD groups (p = 0.028).
Changes in average daily diet intake and physical activity

The dietary intake results are presented in Table 5. In terms of the composition of major nutrients and the number of calories consumed, the WD and CRD groups exhibited good dietary compliance, with no significant difference between the groups. The dietary intakes were significantly lower in the WD and CRD groups than in the MRD group (calories, $p = 0.002$; CHO, $p = 0.001$; protein, $p < 0.0001$). The daily intake of dietary fiber in the MRD group was $12.4 \pm 3.0$ g (8.3 g/1,000 kcal), which was significantly lower than that of the WD group ($22.1 \pm 2.4$ g, 18.7 g/1,000 kcal, $p < 0.001$) or the CRD group ($15.7 \pm 2.3$ g, 12.9 g/1,000 kcal, $p < 0.0001$). The daily intake of cholesterol was significantly lower in the WD group than in the CRD and MRD groups ($p < 0.001$). The intakes of antioxidant vitamins such as vitamin A ($p < 0.001$), vitamin E ($p = 0.004$), beta-carotene ($p < 0.0001$), and vitamin C ($p < 0.0001$) were significantly higher in the WD group than in the CRD and MRD groups. The daily intake of folic acid was significantly higher in the CRD group than in the WD and MRD groups ($p = 0.007$), while the daily intakes of vitamins B$_1$ ($p = 0.002$) and B$_2$ ($p = 0.002$) were significantly higher in the MRD group than in the WD and CRD groups. Physical activity surveys revealed that the body activity (MET) of the CRD and MRD groups tended to decrease during the study that there was no significant difference among the groups ($p > 0.05$).

Safety of the study subjects

During the period of participation in this study, there were no clinically significant changes in the results of diagnostic examinations, vital signs tests, biochemical markers (hematological test, blood biochemistry test, and urine test), and electrocardiograms among the safety parameters ($p > 0.05$) (Supplementary Table 3).

Discussion

To the best of our knowledge, no rigorous clinical studies of detox diets have been conducted. However, this study was conducted as a pilot RCT study to assess the detoxification of toxic trace element following four-week intake of the WD and to verify the efficacy and safety of this diet in reducing body fat in overweight young women. Recently, POPs and heavy metals can accumulate in fat and cause health problems, especially abdominal obesity, which is used as an index of metabolic syndrome [37, 38]. Since heavy metals have very long half-lives, so the process of reduction
(detoxification) of heavy metals that have accumulated in the body is known to be very slow process [39]. Nevertheless, we have performed a rigorous trial to determine the weight loss and detoxification effects. Despite the short-term nature of the present study (four weeks), the levels of eight of the 29 measured heavy metals (lead, nickel, rhodium, uranium, tin, gallium, silver, and tungsten) were reduced or displayed a decreasing tendency in the WD group after the diets (p < 0.1), and four of these eight items (nickel, rhodium, tin, and gallium) were clearly lower in the WD group than in the control diet groups (CRD and MRD). In contrast, we did not observe any significant reduction in heavy metal levels in the hair of subjects in the MRD group. The decreases in some of the heavy metals in the WD group may have been due to removal of diets to the total body burden of toxicants during the intervention period. Also, it is believed that the mediate that improved action of the phytonutrients and micronutrients included in the WD, the intake of dietary fiber and antioxidant vitamins, and the intake of organic foods produced. According to Cline (2015) [40], reactive oxygen species are also a by-product of phase I liver detoxification activity. After going through the phase I processes liver detoxification, the activated toxicants are often more toxic than their parent compounds. If these activated, intermediate metabolites are not further metabolized via the phase II conjugation liver detoxification pathways. Therefore, to quench the propagation of free-radical activity, adequate protection from antioxidant nutrients is required using a number of plant derivatives. Anthropometric indexes were significantly improved in both the WD and CRD groups compared to the MRD group. However, the CRD group lost significantly more weight than the WD and MRD groups, possibly due to the decrease in CHO intake and the subsequent oxidation of fatty acids in subcutaneous tissues. During this study, there was no difference in calorie intake between the WD and CRD groups. It is believed that the major factor that improved the anthropometric indexes in these groups was the significant decrease in their calorie intake compared to the MRD group. Similar to our research, a study by Metzner et al (2011)[41] indicated that the effect of a conventional energy-restricted modified diet (1,200 kcal/d) with or without meal replacement intervention strategies in overweight women had a similar effect on weight loss and body fat distribution but rate of weight loss responder was significantly higher in energy-restricted modified diet group. In general,
the acetyl CoA produced by incomplete oxidation of fatty acids in the liver produces large amounts of ketone bodies such as β-hydroxybutyrate, acetoacetic acid, and acetone. In fact, the urinary β-hydroxybutyrate level in the CRD group was significantly greater after 4 weeks of the diet (p = 0.0034). This may have been related to abnormalities in glucose metabolism. According to Kossoff and Dorward (2008) [42], a reduced blood glucose level due to long-term CHO deficiency is known to cause body protein degradation, similar to what was observed in the CRD group in this study. Also, the large decrease in fat-free mass in the CRD group indicates that the weight loss caused by dietary control was accompanied by reduced muscle mass. Recently Yurrita et al (2017) [26] have reported that significant decreases in body weight, BMI and body fat percentage and greater loss of musculature were observed in the detox diet group (based on juices for 3 days, followed by a hypocaloric diet for 4 days) than in Mediterranean diet group (a hypocaloric Mediterranean diet for 7 days). A study by Lee et al (2017) [43] indicated that whole grain cereal effectively attenuates obesity-related skeletal muscle atrophy as well as overall obesity in obese mice. Because of supplementation of whole grain cereal, which contains of beneficial minerals, vitamins, and other phytochemicals, it suggests that may have helped not only suppress muscle atrophy but also weight loss by activating the phosphatidylinositol 3-kinase and protein kinase B pathway. Therefore, the different effects of the WD and CRD on body fat, despite the similar compositions of these diets (i.e., the proportion of calories and the large amounts of nutrients), suggest that the phytochemicals and micro nutrients included in the WD may have been responsible for the milder reduction of fat-free mass (muscles) in this group. Analysis of safety indicators following WD and CRD supplementation indicated that any changes were within the normal range and had no clinical significance. The strengths of this study are as follows. First, we strictly monitored dietary intake by directly providing all meals to our subjects for four weeks and asking them to complete accurate dietary intake investigated. Second, we confirmed objectively that consuming the WD is superior to consuming calorie-restricted diet in detoxification of heavy metals by comparing and evaluating the effects of a healthy meals based on organic plant based diets consumed in daily life rather than considering the intake of only fruits, vegetables, ingredients or beverages (tea, vinegar, lemon juice, salt water, or drinks mixed with micronutrients).
Also, a more sophisticated assessment was made by investigating the effectiveness of nutritionally balanced detox diets for weight loss and toxin elimination. However, there are some limitations to this study that should be considered. First, four-week intake of this diet was found to reduce certain toxic elements (heavy metals) in hair compared to the control group, although there were limitations in determining the superiority of the test or control diet due to the nature of typical tests for verifying statistical hypotheses. Second, an intake period of long term would be required to verify the efficacy of the diet based measure for discharging heavy metals in hair. Third, the assessment of detoxification of heavy metals and POPs requires an integrated analysis of urine as well as hair analysis. Therefore, we would expect to observe more pronounced detox improvements in a corroborative study with a greater number of subjects and a longer follow-up period.

Conclusions
In conclusion, our results suggest that the WD detox program might have several beneficial effects and safety such as body fat reduction and improving some the element detoxification through caloric restriction. This study lays the scientific foundation for a subsequent large-scale corroborative human study.

Abbreviations
WD: Wellnessup diet CRD: calorie-restricted diet MRD: maintaining regular diet BMI: body mass index LCD: very low-calorie diet POPs: persistent organic pollutants NCDs: non-communicable diseases WC: waist circumference HC: hip circumference WHR waist-to-hip ratio MET: metabolic equivalent task

Declarations

Ethics approval and consent to participate
The study was conducted according to the Declaration of Helsinki. The research protocol was approved by the Institutional Review Board (IRB) of Chonbuk National University Hospital. All participants gave written informed consent.

Authors’ contributions
All authors participated in this work with their substantive contributions. SJJ, WRK, BHP, and SWC carried out the manuscript preparation and statistical analysis, WRK, SJJ, and SWC had the responsibility of data collection and interaction with subjects for the RCT projects. Methology, SJJ, BHP,
SOL, and SWC had participated in the biochemical analysis, interpretation of the data, and review of the paper. The manuscript has been read and approved by all of the authors.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available to protect patient confidentiality but are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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### Tables

**Table 1.** Provided mean dietary contents of the wellness diet group (per day)

| Diets        | WD (Wellness-up diet) | CRD (calorie-restricted diet) |
|--------------|------------------------|-------------------------------|
|              | Breakfast              | Lunch | Dinner | Snack | Total | Breakfast | Lunch | Dinner | Snack | Total |
| Nutri shake  | 58                     | 450   | 400    | 60    | 1,225 | 50        | 450   | 400    | 60    | 1,215 |
| (6 kinds of whole food in a powder) | | | | | | | | | | |
| Mixed fruit and vegetable juice | 274.8 | 246.2 | 509.4 | 194.3 | 1,225 | 245.0 | 266.0 | 509.4 | 194.3 | 1,215 |
| Salad (organic ingredients) | 49.5 | 70.2 | 35 | 13.9 | 177.6 (55%) | 46.0 | 60.5 | 35 | 13.9 | 155.4 (51%) |
| Fruit and nut-based food bars | 6.4 | 4.6 | 22.7 | 5.7 | 39.4 (12%) | 7.0 | 2.7 | 22.7 | 5.7 | 38.1 (13%) |
| Shake (commercial yogurt powder) | 3.2 | 0.6 | 31 | 12.9 | 47.7 (33%) | 3.6 | 1.0 | 31 | 12.9 | 48.5 (36%) |
| Fruit and vegetable juice (commercial) | | | | | | | | | | |
| (commercial yogurt powder) | | | | | | | | | | |
| (commercial ingredients) | | | | | | | | | | |
| (commercial food bars) | | | | | | | | | | |
| (commercial ingredients) | | | | | | | | | | |
| (commercial food bars) | | | | | | | | | | |
| Weight (g) | 58 | 450 | 400 | 60 | 1,225 | 50 | 450 | 400 | 60 | 1,215 |
| Energy (kcal) | 274.8 | 246.2 | 509.4 | 194.3 | 1,225 | 245.0 | 266.0 | 509.4 | 194.3 | 1,215 |
| CHO (g) | 49.5 | 70.2 | 35 | 13.9 | 177.6 (55%) | 46.0 | 60.5 | 35 | 13.9 | 155.4 (51%) |
| Protein (g) | 6.4 | 4.6 | 22.7 | 5.7 | 39.4 (12%) | 7.0 | 2.7 | 22.7 | 5.7 | 38.1 (13%) |
| Fat (g) | 3.2 | 0.6 | 31 | 12.9 | 47.7 (33%) | 3.6 | 1.0 | 31 | 12.9 | 48.5 (36%) |
| Fiber (g) | 3 | 13 | 7.1 | 3.7 | 26.8 | - | 9.0 | 7.1 | 3.7 | 19.8 |

Abbreviations: WD, Wellness-up diet; CRD, calorie-restricted diet;
### Table 2. General characteristics of the subjects

| Variables                  | WD (n=15) | CRD (n=15) | MRD (n=15) | p-value<sup>1</sup> (W-M-C) | p-value<sup>2</sup> (W-M) | p-value<sup>3</sup> (W-C) | p-value<sup>4</sup> (M-C) |
|----------------------------|-----------|------------|------------|-----------------------------|--------------------------|--------------------------|--------------------------|
| Age, years                 | 25.73±4.18 | 24.93±4.57 | 27.93±5.04 | 0.261                       | 0.602                    | 0.234                    | 0.138                    |
| Sex                       | 0/15      | 0/15       | 0/15       | -                           | -                        | -                        | -                        |
| Height (cm)                | 163.33±5.47 | 161.80±5.05 | 162.93±6.65 | 0.683                       | 0.347                    | >0.99                    | 0.517                    |
| Weight (kg)                | 70.21±8.41 | 67.84±6.29 | 70.18±6.59 | 0.687                       | 0.534                    | >0.99                    | 0.407                    |
| Body Mass Index (kg/m<sup>2</sup>) | 26.25±1.98 | 25.91±1.95 | 26.41±1.60 | 0.455                       | 0.507                    | 0.470                    | 0.280                    |
| Body Fat Mass (kg)         | 25.05±4.67 | 23.69±4.48 | 25.18±4.69 | 0.547                       | 0.361                    | 0.803                    | 0.361                    |
| Percent Body Fat (%)       | 35.89±3.42 | 35.25±4.00 | 36.04±4.36 | 0.586                       | 0.455                    | 0.724                    | 0.361                    |
| Waist Circumference (cm)   | 88.46±7.84 | 85.67±7.53 | 87.51±6.14 | 0.633                       | 0.372                    | 0.884                    | 0.493                    |
| Hip Circumference (cm)     | 101.49±3.50 | 99.75±3.48 | 101.87±4.66 | 0.334                       | 0.254                    | 0.884                    | 0.178                    |
| Drinking (yes/no)<sup>5</sup> | 8/7      | 6/9        | 7/8        | 0.765<sup>3</sup>           | 0.464                    | 0.715                    | 0.713                    |
| Alcohol (units/week)       | 2.51±0.53 | 2.55±2.62  | 4.70±3.19  | 0.287                       | 0.212                    | 0.415                    | 0.247                    |
| Current Smoker (yes/no)    | 0/15      | 0/15       | 0/15       | -                           | -                        | -                        | -                        |
| TC (mg/dL)                 | 196.93±32.84 | 182.13±29.34 | 207.27±38.31 | 0.213                       | 0.198                    | 0.561                    | 0.115                    |
| TG (mg/dL)                 | 96.13±44.13 | 74.07±27.14 | 107.20±64.91 | 0.215                       | 0.171                    | 0.709                    | 0.120                    |
| HDL-C (mg/dL)              | 44.54±4.51 | 43.13±4.00  | 44.33±4.03  | 0.713                       | 0.756                    | 0.819                    | 0.361                    |
| LDL-C (mg/dL)              | 88.46±7.84 | 85.67±7.53 | 87.51±6.14 | 0.633                       | 0.372                    | 0.884                    | 0.493                    |
| Glucose (mg/dL)            | 92.20±11.19 | 85.00±5.20  | 86.07±7.41  | 0.083                       | 0.038                    | 0.092                    | 0.819                    |
| Insulin (μU/mL)            | 11.80±4.49 | 9.29±4.13   | 12.14±8.46  | 0.230                       | 0.115                    | 0.299                    | 0.407                    |
| HbA1c (%)                  | 5.41±0.21 | 5.29±0.21   | 5.37±0.36   | 0.430                       | 0.177                    | 0.564                    | 0.596                    |
| hs-CRP (mg/L)              | 18.00±2.99 | 9.61±1.87   | 9.95±1.48   | 0.317                       | 0.183                    | 0.443                    | 0.319                    |
| GGT (IU/L)                 | 27.87±48.53 | 10.00±4.21  | 14.53±7.49  | 0.184                       | 0.191                    | 0.983                    | 0.069                    |
| Uric acid (mg/dL)          | 5.21±1.08 | 4.74±1.06   | 5.13±0.82   | 0.264                       | 0.152                    | 0.934                    | 0.184                    |

Values are presented as mean ± SD.

1) Analyzed by the Kruskal-Wallis test for change in WD-MRD-CRD groups and post-hoc Bonferroni correction. Different letters indicate statistically significant differences between groups.

2) Analyzed by the Wilcoxon rank-sum test for change in WD-MRD groups.

3) Analyzed by the Wilcoxon rank-sum test for change in WD-CRD groups.

4) Analyzed by the Wilcoxon rank-sum test for change in MRD-CRD groups.

5) Analyzed by the Chi-square test.

Abbreviations: WD, Wellnessup diet; MRD, maintaining regular diet; CRD, calorie-restricted diet; TC, Total Cholesterol; TG, Triglycerides; HDL-C, HDL-Cholesterol; LDL-C, LDL-Cholesterol; GGT, Gamma-Glutamyl Transferase.

### Table 3. Changes in harmful elements in hair (units: μg/g)

| Variables | WD (n=15) | CRD (n=15) | MRD (n=15) | p-value<sup>2</sup> (W-M-C) | p-value<sup>3</sup> (W-M) | p-value<sup>4</sup> (W-C) | p-value<sup>5</sup> (M-C) |
|-----------|-----------|------------|------------|-----------------------------|--------------------------|--------------------------|--------------------------|
| Pb        |           |            |            |                             |                          |                          |                          |
| Element | Baseline | 4-week | p-value |
|---------|----------|--------|---------|
| Ni      | 0.59 ± 0.27 | 0.64 ± 0.36 | 0.42 ± 0.24 | 0.668 | 0.443 | 0.819 | 0.547 |
| 4-week  | 0.43 ± 0.33 | 0.64 ± 0.36 | 0.42 ± 0.24 | 0.668 | 0.443 | 0.819 | 0.547 |
| Be      | 0.65 ± 0.27 | 0.64 ± 0.36 | 0.42 ± 0.24 | 0.668 | 0.443 | 0.819 | 0.547 |
| 4-week  | 0.43 ± 0.33 | 0.64 ± 0.36 | 0.42 ± 0.24 | 0.668 | 0.443 | 0.819 | 0.547 |
| As      | 0.0001 ± 0.0003 | 0.0001 ± 0.0001 | 0.0001 ± 0.0001 | 0.0001 ± 0.0001 | 0.0001 ± 0.0001 | 0.0001 ± 0.0001 | 0.0001 ± 0.0001 |
| 4-week  | 0.0000 ± 0.0000 | 0.0000 ± 0.0000 | 0.0000 ± 0.0000 | 0.0000 ± 0.0000 | 0.0000 ± 0.0000 | 0.0000 ± 0.0000 | 0.0000 ± 0.0000 |
| Hg      | 0.53 ± 0.27 | 0.65 ± 0.38 | 0.50 ± 0.24 | 0.668 | 0.443 | 0.819 | 0.547 |
| 4-week  | 0.43 ± 0.33 | 0.64 ± 0.36 | 0.42 ± 0.24 | 0.668 | 0.443 | 0.819 | 0.547 |
| U       | 0.11 ± 0.05 | 0.11 ± 0.08 | 0.15 ± 0.18 | 0.063 | 0.228 | 0.025 | 0.212 |
| 4-week  | 0.06 ± 0.05 | 0.08 ± 0.05 | 0.13 ± 0.10 | 0.063 | 0.228 | 0.025 | 0.212 |
| Sn      | 0.668 | 0.443 | 0.819 | 0.547 |
| 4-week  | 0.43 ± 0.33 | 0.64 ± 0.36 | 0.42 ± 0.24 | 0.668 | 0.443 | 0.819 | 0.547 |
| Cd      | 0.004 ± 0.004 | 0.005 ± 0.005 | 0.004 ± 0.004 | 0.004 ± 0.004 | 0.004 ± 0.004 | 0.004 ± 0.004 | 0.004 ± 0.004 |
| 4-week  | 0.005 ± 0.005 | 0.004 ± 0.004 | 0.005 ± 0.004 | 0.004 ± 0.004 | 0.004 ± 0.004 | 0.004 ± 0.004 | 0.004 ± 0.004 |
| Pd      | 0.104 | >0.999 | 0.703 |
| 4-week  | 0.0002 ± 0.0002 | 0.0002 ± 0.0002 | 0.0003 ± 0.0003 | 0.0002 ± 0.0002 | 0.0002 ± 0.0002 | 0.0003 ± 0.0003 | 0.0002 ± 0.0002 |
| Gd      | 0.0003 ± 0.0001 | 0.0002 ± 0.0002 | 0.0003 ± 0.0003 | 0.0002 ± 0.0002 | 0.0002 ± 0.0002 | 0.0003 ± 0.0003 | 0.0002 ± 0.0002 |
| Element | Baseline | 4-week | p-value |
|---------|----------|--------|---------|
| Ga      | 0.00017±0.00010 | 0.0012±0.00004 | 0.042 0.213 0.014 0.198 |
| p-value | 0.139 0.064 | >0.999 0.804 | 0.945 0.135 |
| Au      | 0.011±0.019 | 0.010±0.011 |   |
| p-value | 0.313 0.847 |   0.234 |
| Rb      | 0.0787±0.093 | 0.0737±0.081 |   |
| p-value | 0.825 0.847 |   >0.999 |
| Ba      | 1.530±1.3132 | 1.399±0.9944 |   |
| p-value | 0.847 0.313 |   >0.999 |
| Pt      | 0.0001±0.0001 | 0.0000±0.0001 |   |
| p-value | 0.531 0.847 |   0.208 |
| Bi      | 0.0118±0.0262 | 0.0062±0.0063 |   |
| p-value | 0.248 0.531 |   >0.999 |
| Ce      | 0.0053±0.0073 | 0.0049±0.0047 |   |
| p-value | 0.463 0.463 | 0.552 0.552 |
| Cs      | 0.0004±0.0007 | 0.0005±0.0007 |   |
| p-value | 0.625 0.625 | 0.500 0.500 |
| Sb      | 0.011±0.0077 | 0.012±0.008 |   |
| p-value | 0.352 0.352 | 0.352 0.352 |
| Al      | 3.49±1.6534 | 3.66±1.8658 |   |
| p-value | 0.934 0.934 | 0.367 0.367 |
| Ag      | 0.116±0.1677 | 0.080±0.0105 |   |
| p-value | 0.092 0.092 | 0.561 0.561 |
| In      | 0.00±0.00 | 0.00±0.00 |   |
| p-value | 0.398 >0.999 | 0.351 0.351 |
| Zr      | - - | - - | - - |
|       | Baseline        | 4-week          | 4-week          | 4-week          |
|-------|-----------------|-----------------|-----------------|-----------------|
|       | 0.017±0.01      | 0.025±0.03      | 0.009±0.00      | 0.017±0.01      |
| W     | 2               | 5               | 4               | 9               |
| p-value| 0.382           | 0.717           | 0.133           |
|       | 0.014±0.00      | 0.025±0.03      | 0.009±0.00      | 0.017±0.01      |
| W     | 8               | 5               | 4               | 9               |
| p-value| 0.670           | 0.560           | 0.724           | 0.418           |
|       | 0.0023±0.00     | 0.0041±0.00     | 0.0023±0.00     | 0.0023±0.00     |
| W     | 011             | 051             | 012             |
| p-value| 0.217           | 0.633           | 0.158           | 0.135           |
|       | 0.0015±0.00     | 0.0020±0.00     | 0.0022±0.00     | 0.0022±0.00     |
| W     | 008             | 037             | 024             |
| p-value| 0.002           | 0.015           | 0.337           |
|       | 0.0006±0.00     | 0.0040±0.00     | 0.0001±0.00     | 0.0001±0.00     |
| Tc    | 014             | 003             | 002             |
| p-value| 0.270           | 0.754           | 0.134           | 0.228           |
|       | 0.0005±0.00     | 0.0030±0.00     | 0.0001±0.00     | 0.0001±0.00     |
| Tc    | 011             | 003             | 001             |
| p-value| 0.793           | 0.858           | 0.571           |

Values are presented as mean ± SD or number.

1) Analyzed by the Wilcoxon signed-rank test.

2) Analyzed by the Repeated Measures ANOVA according to the satisfaction of normality.

3) Analyzed by the Wilcoxon rank-sum test for change in WD-MRD groups.

4) Analyzed by the Wilcoxon rank-sum test for change in WD-CRD groups.

5) Analyzed by the Wilcoxon rank-sum test for change in MRD-CRD groups.

Abbreviations: WD, Wellnessup diet; MRD, maintaining regular diet; CRD, calorie-restricted diet
### Table 4. Changes in harmful elements in hair (units: μg/g)

| Variables                      | WD (n=15)       | CRD (n=15)      | MRD (n=15)      | p-value<sup>1)</sup> (W-M-C) | p-value<sup>2)</sup> (W-M) | p-value<sup>3)</sup> (W-C) | p-value<sup>4)</sup> (M-C) |
|--------------------------------|-----------------|-----------------|-----------------|------------------------------|----------------------------|----------------------------|----------------------------|
| **Weight (kg)**                |                 |                 |                 |                              |                            |                            |                            |
| Baseline                       | 69.59±8.98      | 66.82±6.59      | 69.51±6.97      |                              |                            |                            |                            |
| 4-week                         | 67.71±9.93      | 63.60±6.11      | 69.29±6.98      | 0.001                        | 0.0125                     | 0.050                      | 0.001                      |
| p-value<sup>1)</sup>           | 0.013           | 0.001           | 0.322           |                              |                            |                            |                            |
| **Body Mass Index (kg/m<sup>2</sup>)** |                 |                 |                 |                              |                            |                            |                            |
| Baseline                       | 26.02±2.27      | 25.50±2.12      | 26.15±1.83      |                              |                            |                            |                            |
| 4-week                         | 25.30±2.72      | 24.27±1.99      | 26.07±1.89      | 0.004                        | 0.164                      | 0.046                      | 0.001                      |
| p-value<sup>1)</sup>           | 0.011           | 0.001           | 0.296           |                              |                            |                            |                            |
| **Body Fat Mass (kg)**         |                 |                 |                 |                              |                            |                            |                            |
| Baseline                       | 44.54±5.51      | 43.13±3.70      | 44.33±4.03      |                              |                            |                            |                            |
| 4-week                         | 43.45±5.97      | 41.05±3.55      | 44.16±3.86      | 0.001                        | 0.046                      | 0.019                      | 0.001                      |
| p-value<sup>1)</sup>           | 0.002           | 0.001           | 0.366           |                              |                            |                            |                            |
| **Waist Circumference (cm)**   |                 |                 |                 |                              |                            |                            |                            |
| Baseline                       | 88.46±7.84      | 85.67±7.53      | 87.51±6.14      |                              |                            |                            |                            |
| 4-week                         | 85.80±8.81      | 81.40±6.35      | 86.64±6.13      | 0.009                        | 0.184                      | 0.135                      | 0.008                      |
| p-value<sup>1)</sup>           | 0.007           | 0.001           | 0.367           |                              |                            |                            |                            |
| **Hip Circumference (cm)**     |                 |                 |                 |                              |                            |                            |                            |
| Baseline                       | 101.49±3.50     | 99.75±3.48      | 101.87±4.66     |                              |                            |                            |                            |
| 4-week                         | 99.87±4.36      | 97.35±3.93      | 101.64±5.05     | 0.006                        | 0.361                      | 0.040                      | 0.002                      |
| p-value<sup>1)</sup>           | 0.010           | 0.001           | 0.366           |                              |                            |                            |                            |
| **Waist to Hip Ratio**         |                 |                 |                 |                              |                            |                            |                            |
| Baseline                       | 0.87±0.05       | 0.86±0.07       | 0.86±0.05       |                              |                            |                            |                            |
| 4-week                         | 0.86±0.06       | 0.84±0.06       | 0.85±0.05       | 0.233                        | 0.393                      | 0.303                      | 0.138                      |
| p-value<sup>1)</sup>           | 0.050           | 0.009           | 0.473           |                              |                            |                            |                            |

Values are presented as mean ± SD or number.

1) Analyzed by the Wilcoxon signed-rank test
2) Analyzed by the Repeated Measures ANOVA according to the satisfaction of normality.
3) Analyzed by the Wilcoxon rank-sum test for change in WD-MRD groups.
4) Analyzed by the Wilcoxon rank-sum test for change in WD-CRD groups.
5) Analyzed by the Wilcoxon rank-sum test for change in MRD-CRD groups.

Abbreviations: WD, Wellnessup diet; MRD, maintaining regular diet; CRD, calorie-restricted diet

### Table 5. Mean nutrient intakes of the study subjects during the 4-week intervention period

| Variables      | WD (n=15)       | CRD (n=15)      | MRD (n=15)      | P-value<sup>1)</sup> (W-M-C) | P-value<sup>2)</sup> (W-M) | P-value<sup>3)</sup> (W-C) | P-value<sup>4)</sup> (M-C) |
|----------------|-----------------|-----------------|-----------------|------------------------------|----------------------------|----------------------------|----------------------------|
| **Energy (kcal)** |                 |                 |                 |                              |                            |                            |                            |
| Baseline       | 1,521.07±440.37 | 1,894.64±623.99 | 1,684.41±34.94 |                              |                            |                            |                            |
| 4-week         | 1,184.76±162.72 | 1,217.80±232.21 | 1,495.36±31.01 | 0.002                        | 0.481                      | 0.002                      | 0.003                      |
|                |                 |                 |                 |                              |                            |                            |                            |
| **Carbohydrate (g)** |                 |                 |                 |                              |                            |                            |                            |
| Baseline       | 218.75±71.942   | 260.50±103.781  | 228.63±62.58    |                              |                            |                            |                            |
| 4-week         | 158.31±21.50a   | 151.58±31.29a   | 198.99±44.41b   | 0.001                        | 0.213                      | 0.003                      | 0.001                      |
|                |                 |                 |                 |                              |                            |                            |                            |
| **Fat (g)**    |                 |                 |                 |                              |                            |                            |                            |
| Baseline       | 47.20±18.63     | 62.25±23.35     | 57.18±14.03     |                              |                            |                            |                            |
| 4-week         | 48.52±6.32      | 50.98±8.80      | 50.30±12.02     | 0.971                        | 0.772                      | 0.648                      | 0.967                      |
| Parameter                  | 4-week          | Baseline       | p-values          |
|---------------------------|-----------------|----------------|------------------|
| **Cholesterol (mg)**      |                 |                |                  |
| Baseline                  | 248.06±126.65   | 356.29±149.75  | <0.0001 0.075    |
| 4-week                    | 70.04±48.74     | 89.95±47.64    | <0.0001 0.110    |
| **Fiber (g)**             |                 |                |                  |
| Baseline                  | 11.88±3.86      | 16.07±6.67     | <0.0001 0.075    |
| 4-week                    | 22.11±2.40a     | 15.73±2.34b    | <0.0001 0.002    |
| **Calcium (mg)**          |                 |                |                  |
| Baseline                  | 377.43±195.98   | 446.34±131.03  | 0.001 0.009      |
| 4-week                    | 424.59±54.72b   | 334.63±51.30a  | <0.0001 <0.0001  |
| **Phosphorus (mg)**       |                 |                |                  |
| Baseline                  | 764.90±189.2    | 983.31±310.3   | 0.001 0.047      |
| 4-week                    | 634.49±96.12ab  | 71.84±114.25ab | 0.001 0.008      |
| **Potassium (mg)**        |                 |                |                  |
| Baseline                  | 1,695.20±412.54 | 2,187.72±962.5 | 0.019 0.481      |
| 4-week                    | 1,940.69±283.46 | 1,951.69±276.5 | 9.032 0.159      |
| **Zinc (mg)**             |                 |                |                  |
| Baseline                  | 7.70±1.97       | 9.25±2.55      | 0.0001 0.025     |
| 4-week                    | 5.48±0.98a      | 6.41±1.12a     | <0.0001 <0.0001  |
| **Vitamin A (RE)**        |                 |                |                  |
| Baseline                  | 583.42±345.42   | 623.20±280.04  | 0.001 0.038      |
| 4-week                    | 772.80±98.58b   | 386.11±79.30a  | 0.0001 <0.0001   |
| **Vitamin E (mg)**        |                 |                |                  |
| Baseline                  | 14.43±4.74b     | 13.44±5.15ab   | 0.004 0.002      |
| 4-week                    | 13.11±1.63b     | 12.26±2.14ab   | <0.0001 0.147    |
| **Vitamin C (mg)**        |                 |                |                  |
| Baseline                  | 71.25±58.85     | 93.84±75.05    | 0.844 0.804      |
| 4-week                    | 210.70±19.63b   | 202.30±27.91b  | <0.0001 0.263    |
| **β-carotene (mg)**       |                 |                |                  |
| Baseline                  | 2,662.55±2,166.65 | 2,509.21±1,362.01 | 2,653.95±1,796.33 |
| 4-week                    | 4,438.30±494.09b | 2,078.55±405.41a | 2,049.79±82.149a |
| **Vitamin B1 (mg)**       |                 |                |                  |
| Baseline                  | 1.06±0.43       | 1.17±0.31      | 0.002 0.002      |
| 4-week                    | 0.84±0.11ab     | 0.88±0.12a     | 0.002 0.005      |
| **Vitamin B2 (mg)**       |                 |                |                  |
| Baseline                  | 1.00±0.39       | 1.21±0.34      | 0.002 0.004      |
| 4-week                    | 0.76±0.11a      | 0.77±0.34a     | 0.002 0.004      |
| **Vitamin B6 (mg)**       |                 |                |                  |
| Baseline                  | 1.10±0.29       | 1.46±0.55      | 0.002 0.005      |
| 4-week                    | 1.15±0.16       | 1.16±0.19      | 0.002 0.004      |
| **Folic acid**            |                 |                |                  |
| (μg) | | | |
|------|---|---|---|
| Baseline | 296.85±68.19 | 372.74±150.30 | 347.81±132.58 |
| 4-week | 347.61±56.94 | 383.58±37.19 | 298.10±86.69 |

**MET value (min/week)**

| | | | |
|------|---|---|---|
| Baseline | 2,209.33±2,397.51 | 1,786.67±1,470.39 | 2,626.67±2,465.61 |
| 4-week | 2,033.33±2,090.20 | 1,373.33±1,243.67 | 977.33±966.37 |

Values are presented as mean ± SD

1) Analyzed by the Repeated Measures ANOVA according to the satisfaction of normality and post-hoc Bonferroni correction. Different letters indicate statistically significant differences between groups.

2) Analyzed by the Mann-Whitney U test for change between W-M.

3) Analyzed by the Mann-Whitney U test for change between W-C.

4) Analyzed by the Mann-Whitney U test for change between M-C.

Abbreviations: WD, Wellnessup diet; MRD, maintaining regular diet; CRD, calorie-restricted diet; MET, metabolic equivalent

Figures
Figure 1

Flow diagram of the participants in this study

Supplementary Files
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Supplementary file 20.02.03.docx