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Article

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Abstract

Purpose The menopausal transition (MT) is a period when there may be an increase in visceral fat mass and a worsening of cardiometabolic risk factors. Few studies have evaluated the efficacy of plant-based low-calorie diets on groups of women at different stages of MT. The aim of this study is to compare the effectiveness of a high plant-to-animal protein ratio diet in women of similar age but with different fertility statuses

Design/methodology/approach Subjects were divided into three groups according to their fertility status at the baseline: ~~REGULAR~~ "pre-menopausal" (n = 11), ~~PERIMENOPAUSE~~ "perimenopause"(n = 14), and ~~MENOPAUSE~~ "post-menopause"(n = 18). Body composition was measured at the beginning and after 8 months of treatment. Individualised lifestyle treatment included a strong component of plant-based foods.

Findings Forty-three overweight or obese Caucasian women (age 52.3 ± 4.5 years, body mass index [BMI] 30.6 ± 5.4 kg/m^2, fat mass 33.1 ± 9.3 kg; data presented as means ± SD) were included in the study. Mean physical activity was 8.4 ± 7.6 METs/week. Subjects had an improvement in body composition (fat mass -5.6 ± 4.0 kg, $p < 0.001$; protein -0.3 ± 0.5 kg, $p < 0.001$), HDL-C and systolic blood pressure values. Waist circumference and hip circumference decreased by 4.1 ± 3.1 cm and -6.0 ± 4.3 cm, respectively. Weight loss resulted in a significant improvement in some blood lipid values, such as total and HDL cholesterol. Adherence to a high plant protein diet helps adult women with different fertility statuses to improve body composition and reduce cardiovascular risk factors. Long-term studies with larger sample sizes are needed to confirm these findings.

Keywords: plant-based diet, menopause, weight loss, body composition, unstructured physical activity

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1. Introduction

Many physical changes occur in women during the menopausal transition (MT). The cyclic secretion of production of female sex hormones such as oestradiol and progesterone declines rapidly, and these hormonal alterations can lead to worsening of body composition (BC) as oestradiol is associated with reduced accumulation of abdominal fat and increased gluteo-femoral fat due to the activity of lipoprotein lipase in femoral adipocytes. [1] The alteration of lipid metabolism has an influence on body composition and various aspects of energy metabolism. [2]

In elderly subjects, a quota of at least 1.5 g/kg of daily protein has been proposed to preserve fat free mass (FFM) loss and reduce the risk of sarcopenic obesity. [3] Consumption of protein from animal sources is suggested by nutritional guidelines because they are complete in terms of essential amino acids. Thus, animal proteins are often considered "high-quality" protein and may reduce the supposed risk of amino acid deficiency, while plant-based protein sources are said to "have lower anabolic potential than animal-based proteins." [4]

In large prospective cohort studies, higher plant protein intake was inversely associated with the risk of overall and cardiovascular disease-associated mortality. [5-6] Replacing animal protein-rich foods with vegetable protein varieties has been linked to longevity. [7] Higher ratios of animal and vegetable proteins in the diet and higher meat intake have been associated with higher mortality in a study that evaluated the dietary habits of approximately 2,600 Finnish men aged between 42 and 60, with an average follow-up of 20 years. [8] Substitution of plant protein for animal protein was associated with lower mortality in a prospective cohort study of US healthcare workers that included 131,342 subjects from the Nurses' Health Study. [6] A healthy diet is inversely associated with cardiovascular mortality and can be used to recommend the type of protein sources, preferring low protein contributions from meat and higher intakes of plant proteins from nuts and seeds. [9]

Proposing a healthy nutritional model from the moment the diet is prescribed could promote long-term compliance. The aim of this study is to evaluate the effectiveness of a diet high in vegetable protein in women of similar age but different fertility status.

2. Materials and Methods

Subjects: Fifty women motivated to lose weight were asked to join the study in 2019–2020. The inclusion criteria were a BMI of at least 25 kg·m-2 but less than 35 kg·m-2, and age between 45 and 60 years. Pregnant or lactating patients and patients on medication that could affect menstrual cycle or weight loss or basal metabolism were excluded. Participants were divided into three groups according to their fertility status at the baseline. The first group ~~REGULAR~~ (pre-menopausal) were women with a regular menstrual cycle. The other two groups were comprised of women who were experiencing some or all of the following symptoms: hot flashes, night sweats, vaginal dryness, mood changes, and insomnia. The second group included women who have not had a period for ~~at least~~ up to 12 months and were not using hormonal contraception (~~PERIMENOPAUSE~~ perimenopause); and the third group (~~MENOPAUSE~~ post-menopause) ~~were women with no menstrual period for more than a year or whose FSH blood level was consistently elevated to ≥30 mIU/mL~~ included women who not had a menstrual period for at least 12 consecutive months and when the follicle-stimulating hormone (FSH) blood level was elevated to 30 mIU/mL or higher. All subjects underwent a complete medical visit, including assessment of anthropometrics, BC and blood pressure. Blood pressure was determined after at least 10 minutes of rest in the sitting position, with an electronic validated sphygmomanometer (Omron M3) on both arms. For all factors, data was taken from two measurements, and mean results were reported. Height was assessed in centimeters with a standard height measuring rod. Waist (WC) and hip circumferences were measured to the nearest 1 cm using an anthropometric tape. All patients were assessed wearing only underwear and with feet about 25–30 cm apart, and measurements were taken parallel to the floor without compressing the skin. WC was evaluated at the midpoint between the iliac crest and the minimal waist. The hip circumferences was assessed around the widest portion of the buttocks with legs and feet together.

Body composition assessment: Weight and BC were assessed after overnight fasting using a Tanita BC-420 MA (TANITA Corporation, Sportlife Tokyo, Japan; range of 0–200 kg, accuracy: 100 g), an instrument validated using dual-X-ray absorptiometry (DXA) [10]. Subjects were asked to follow the following rules before BC evaluation: urination before testing; to avoid the menstruation period; testing at least 3 hours after meals and at least 3 hours after waking up; and no high intensity physical activity within 12 hours before testing. The parameters available are fat mass (FM), FFM, skeletal muscle and hydration status (total body water, TBW).

Dietary intervention: At least one third of the time of the first visit was devoted to nutrition education. In addition to illustrating the basic principles of the diet, the dietician, through the use of nutritional tables, guided the patients in the interpretation of food labels and conscious food shopping. Subjects were asked to weigh foods using home digital scales. During the visit a taste and food habits test was administered to the patients to evaluate their eating habits and taste as described previously. [11]. The proposed nutritional model was individualised according to the subjects' survey results and could include one to three daily snacks based on seasonal fruits, in addition to breakfast and two main meals. The diets were based on the traditional Nicotera's Mediterranean diet, including a strong component of plant-based foods (such as legumes, nuts, seeds, and whole-grain pasta and bread). Proteins of animal origin were present in only one daily meal, usually at lunchtime, and, according to personal preference, at breakfast in the form of milk (150 ml) or white yoghurt (120 g), with no added-sugar industrial products. Among foods of animal origin, people were asked to choose mainly small blue fish (such as mackerel, sardines and anchovies) and limited consumption of white meat, eggs and low-fat cheese. The consumption of red meat and processed meat was not to exceed twice a month. The daily dietary fibre intake was at least 35 g per day. Patients with intestinal discomfort, due to the large amount of legumes and vegetables proposed, were given a 4-week plan with a gradual increase of these foods. A 500–600 kcal/day deficit compared to the estimated total energy expenditure was proposed. A dieting software was used to elaborate the diets (Winfood 2.8; Medimatica Srl, Martinsicuro, Italy).

Physical activity: Patients were provided with personalised physical activity suggestions based on their life habits and previous sports experience, but not with a predefined training plan. The proposed training was mainly aerobic. Based on the physical activity diary, the calculation of weekly average METs was carried out by calculating the time spent per week on each sport activity. Regular moderate and vigorous physical activity was assessed with a validated physical activity questionnaire and converted to weekly METs. [12]

Follow-ups: Every month, women were scheduled to meet the dietician to support the lifestyle intervention programme. A chat service was also provided to answer all questions between the visits. Patients were asked to record their weekly dietary intake in a semi-quantitative weighed food record once a month throughout the follow-up. Dietary composition was analysed from the diaries using professional software (Nutritics, 2018). To avoid underreporting, all patients whose daily intake was <110% of their basal metabolic rate were excluded, as described by Mendez et al. [13] Women were measured using the same BC parameters described above.

Laboratory data: Blood samples were taken between 7:00 AM and 9:00 AM. Assessments of glucose, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and creatinine were measured using standard immune-enzymatic methods. The same haematochemical measurements were repeated after eight months.

Statistical analysis: Statistical analysis was performed using SPSS 24.0 software (SPSS, Chicago). Mean ± SD with 95% CI or percentages were used as descriptive statistics. All quantitative variables were tested for normality of the distribution using the Kolmogorov-Smirnov test. Differences in continuous variables between groups were assessed using the ANOVA test for multiple comparisons. Chi-squared statistics were used to test the sample differences in taste and food habits. Differences in proportions of discrete traits were investigated using Pearson's correlation test. For all analyses, a p-value <0.05, based on a two-sided test, was considered statistically significant.

3. Results

Of the initial 50, seven women were excluded. Three dropped out for personal reasons, one because the reported calories from her diaries were lower than 110% of her basal metabolic rate (BMR) for two consecutive visits, and three subjects did not answer our communications. Mean characteristics of the 43 Caucasian women who were included in the study are shown in Table 1. The total sample had an average age of 52.3 ± 4.5 years, and 17 were smokers. The breakdown into the three groups was as follows: 11 subjects in the ~~REGULAR~~ pre-menopausal group (age 48.2 ± 2.7 years), 14 in the ~~ERIMENOPAUSE~~ perimenopause– group (age 51.5 ± 2.9 years), and 18 in the ~~MENOPAUSE~~ post-menopause group (age 55.5 ± 4.1 years). All participants were overweight or obese; the average BMI was 30.6 ± 5.4 kg/m2, FM was 33.1 ± 9.3 kg, FFM was 45.7 ± 4.9 kg, and TBW was 34.1 ± 4.1 kg. Mean physical activity was 8.4 ± 7.6 METs/week. There were no significant basal differences between the three groups, apart from age ($p < 0.001$) and total cholesterol ($p = 0.04$). The composition of the dietary intake and percentage breakdown among meals are shown by group in Table 2. ~~Micronutrient contents are illustrated in the supplementary material (Table S1).~~ The average macronutrient intake was 0.8 g/kg/day of protein, 32.9% fat and 53.1% carbohydrates. The daily distribution of the diet included three main meals and two or three snacks. ~~The evaluation of the test concerning food and water preferences (Table S2), sleep and food habits (Table S3) and food taste (Table S4) are presented in the supplementary material. showed no differences were found for these parameters between the three study groups.~~ The results of the test regarding food and water preferences, sleep and eating habits and food taste showed no significant differences between the three study groups (data not shown).The changes for all subjects and between groups after 8 months are shown in Table 3. The total sample had a significant improvement in all BC parameters, and HDL and blood pressure. The difference in BMI was -2.9 ± 1.8 kg/m2. The average FM decreased by 5.6 ± 4 kg, and the FFM reduction was 2.0 ± 1.4 kg, of which 1.7 ± 1.2 kg was TBW and 0.3 ± 0.5 kg was body protein. WC decreased by 4.1 ± 3.1 cm, and hip circumference variation was -6.0 ± 4.3 cm. The weight loss allowed a significant improvement in some lipid blood values, such as total cholesterol and HDL, in the whole sample. The mean variation in BMR was 72.3 ± 46.1 kcal/day. There were no significant group differences for any of these factors.

In the whole group, plant to animal protein ratio was directly correlated (Figure 1) with total dietary fibre (Pearson's correlation, r = 0.495; p = 0.001) and polyunsaturated fatty acids (PUFA; r = 0.438; p = 0.005). Plant to animal protein ratio was inversely associated with total dietary protein (r = -0.503; p = 0.001) and SFA (r = -0.434; p = 0.006). Animal protein in the diet was related (Figure 2) to saturated fatty acids (SFA; r = 0.689; p < 0.001) and systolic blood pressure difference (p = 0.023; r = 0.358). Consumption of animal protein was not significantly related to body protein variation (r = 0.14; p = 0.14). Plant protein in the diet was inversely correlated with the variation in BMR (r = -0.334; p = 0.035). Total dietary fibre was correlated (Figure 3) with body protein variation (r = 0.438; p = 0.004), while insoluble fibre was inversely related to WC difference (r = -0.351; p = 0.036). Weekly METs were inversely related (Figure 4) to body protein variation (p = 0.044; r = -0.309), but was not significantly related to FM variation (r = 0.01; p = 0.99).

4. Discussion

Epidemiological studies [14-15] have confirmed that MT is a period when there may be an increase in FM, particularly in the abdomen, with associated exacerbation of health risks. A longitudinal study with yearly measurements for 4 years revealed that middle-aged women gained subcutaneous abdominal fat with age, while menopause was linked to an increase in FM and visceral abdominal fat. This study also showed that FM is related to a reduction in total energy expenditure and fat oxidation, which may lead to obesity. [16] MT is also linked to increased appetite, probably due to the hormonal changes: progesterone and testosterone may stimulate hunger while oestrogens may inhibit food intake. [17] A recent trial has assessed differences in BC and FM distribution in women during the MT. At the beginning of the MT, the rate of FM gain doubled, and lean mass (LM) reduced. The BC changes lasted for 2 years. [18] Weight loss as part of a healthy modification of diet may help to eliminate vasomotor symptoms among postmenopausal women. Dietary intervention appeared to improve symptoms, in addition to the effect of weight change. [19] Although most diets are successful for short-term weight loss, long-term results are generally weak. Therefore, it is relevant to explore which diets are most efficacious for long-term weight management in this target population. [20] We have previously demonstrated that high adherence to the traditional Mediterranean diet might help menopausal women to lose FM and maintain LM, with similar outcomes to younger women. [21] In this new study, we have shown that the MT period does not reduce the effectiveness of weight loss. A diet high in plant-based foods results in highly significantly improved BC in these subjects. MD has been compared to pharmacological interventions in terms of reducing the risk of obesity and cardiovascular and metabolic events. [22] High adherence to MD, avoidance of tobacco, higher consumption of whole grains, nuts, fruits, legumes, whole milk products and olive oil, and lower consumption of sugary drinks could contribute to a healthier BC during MT. [23]

Several papers confirmed the positive effects of a diet with high levels of protein from vegetable sources. A 2018 trial of dietary models in postmenopausal women suggested that the group with high unrefined grain and legume consumption and low refined grain intakes were associated with poorer BMI, while high red meat and potato intakes and low nut and coffee/tea consumption were associated favourably with BMI. These relationships were independent of age, years since menopause, energy intake and physical exercise. [24] The patients in our study were suggested a nutritional model with a high content of vegetable protein. As the diet was individualised and also based on personal preference, there was a fair amount of variability in food choices in relation to animal or vegetable protein. Achieving the 25–30 g of fibre per day suggested by all nutritional guidelines is very difficult [25], especially in the very popular low-calorie diets that involve the total exclusion of foods rich in carbohydrates. Several studies have previously demonstrated beneficial effects of fibre consumption for pre-menopausal women. There was a lower risk of early onset of menopause among women who ate more plant protein foods, equivalent to 3–4 servings per day, but no effect with animal protein. [26] Another study highlighted the beneficial effects of fruit and vegetable consumption in preventing metabolic syndrome, particularly among postmenopausal women. [27]

As shown in figure 1, increasing the amount of plant protein in patients with different fertility statuses allows improvement in dietary fibre intake and PUFA. Higher consumption of vegetable proteins is also inversely related to total diet protein and SFA. Particularly in dyslipidaemic patients, this would allow a lower proportion of SFA in the diet with effects. [28] Even in our group of patients who were prescribed a high plant protein diet, the consumption of animal protein in the diet was associated with lower effects on the variation in systolic blood pressure. This data confirms that the intake of plant protein may prevent the onset of risk factors associated with cardiovascular disease, such as hypercholesterolemia and hypertension. [29] Higher fibre content showed proportionally beneficial effects on body protein and abdominal circumference in our patients (figure 3). One negative effect of higher plant protein in the diet was an increased reduction of BMR. This could be explained by the lower thermal effect induced by plant proteins compared to animal proteins. [4] In our study, however, the higher consumption of plant proteins did not show lower anabolic effects. In addition, plant-based diets are rich in phytoestrogens, which are plant compounds found in some seeds, legumes, and some fruits and vegetables. Phytoestrogens have been shown to mimic the benefits of endogenous estrogens, and diets rich in phytoestrogens are considered promising approaches to alleviate menopausal symptoms [30] and reduce the risk of postmenopausal cardiovascular disease. [31]

The assessment of eating habits and food preferences showed no substantial differences in the three study groups ~~(tables in the supplementary material)~~. Women showed no tendency towards eating disorders or poor food choices, with a good variety of food preferences and the exclusion of foods such as tofu and vegetable drinks. The subjects, even before our nutrition education intervention, showed an interest in an increased consumption of healthy foods, such as high-fibre foods. Positive effects of a healthy diet were observed among the respondents' results for Polish women aged 50–60. In the healthy diet group, fruit, vegetables and wholemeal bread were the most frequently consumed products. Among unhealthy foods, women chose sweets, cheese and fried dishes most often. [32] In Spanish women over 40, no association was observed between dietary habits and the age of natural menopause. [33]

Our results suggest that increasing weekly METs would not provide any additional benefits beyond those of diet on BC in women at different stages of MT (figure 4). MT leads to a reduction in TEE mostly due to a decrease in energy expenditure during physical activity, caused mainly by a more sedentary lifestyle. [34] Our study agrees with another paper that demonstrated that light physical activity may have a greater impact on adiposity than moderate and/or vigorous physical activity, independent of the menopausal status. [35]

Our study has several limitations. The study sample was not particularly large and there was a difference in group sizes, with the group of fertile women being smaller than the group of menopausal women. The methods for assessing BC and BMR should have been more accurate than those used. It was not possible to repeat the lifestyle test at the end of the study, which would have allowed us to also assess the effects of the lifestyle intervention on eating habits and tastes.

5. Conclusions

Adherence to a high plant protein diet helps adult women with different fertility statuses to improve body composition and reduce cardiovascular risk factors. Studies demonstrating the long-term effects of diet therapies to promote long-term weight management are needed.

Abbreviations

BC: Body Composition; BMR: basal metabolic rate; FM: fat mass; FFM: fat-free mass; LM: lean body mass; MET:Metabolic Equivalent of Task; MT: Menopausal Transition; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; TBW: total body water; WC: waist circumference

~~**Supplementary Materials:** The following are available online at *********. Table S1: Differences in micronutrient content of study group diets, Table S2: Differences between groups on food and water preferences, Table S3: Differences between groups in regard to the relationship with meals and sleeping habits, Table S4: Differences in food taste between groups~~

Author Contributions: M.L., G.R. and A.B. performed the literature search, participated in the data collection and analyses, and drafted the manuscript; A.F. and M.A.P. helped in the final revision of the paper; M.C., C.B., D.L. and E.P. participated in the design of the study and drafted the manuscript; M.L. was the principal investigator of the study, led its design, coordinated the steps of the data collection and data analyses, and drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by Institutional Research Board (School of Sports and Exercise Science, University of Rome "Tor Vergata", Faculty of Medicine and Surgery, protocol number ITPro2016-0004). All participants could choose if they wanted to participate in the study and could at any time withdraw their consent.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data used in this manuscript are publicly available from previous publications and fully disclosed in the Tables of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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Table 1: Body composition and other characteristics of study subjects.

	~~Regular~~ pre-menopausal (n=11)	perimenopause (n=14)	~~Menopause~~ post-menopause (n=18)	*p*	Total (n=43)
Age, y	48.2±2.7	51.5±2.9	55.5±4.1	<.0011	52.3±4.5
Smokers, n	0	1	5		6
Height, m	162.5±5.1	164.9±7.3	160.7±6.3	0.19	162.6±6.5
Body Weight, kg	84.2±15.9	78.2±7.9	80.5±15.6	0.55	80.7±13.5
BMI, kg/m^2	31.9±5.6	28.9±3.7	31.3±6.3	0.32	30.6±5.4
FM, kg	36.3±10	30.8±6.0	32.9±10.7	0.34	33.1±9.3
FFM, kg	47.2±5.4	45.0±3.4	45.3±5.5	0.48	45.7±4.9
TBW, kg	35.5±4.6	33.5±2.7	33.7±4.7	0.43	34.1±4.1
Body Protein, kg	11.8±0.9	11.5±0.7	11.6±1.0	0.80	11.6±0.9
Waist, cm	100.5±10.4	98.5±6.1	103.8±17.1	0.51	101.2±12.6
Hip, cm	114.9±10.2	109.4±6.2	111.5±12.8	0.43	111.7±10.3
BMR, kcal	1528.2188.3±	1450.1±95.1	1457.4±184.3	0.43	1473.1±161.4
SBP, mmHg	123.2±18.7	130.8±14.6	128.8±11.3	0.42	128.0±14.5
DBP, mmHg	83.5±11.1	82.1±9.3	79.1±6.6	0.39	81.2±8.8
MET, METs/wk	10.4±8.7	5±4.4	9.8±8.2	0.12	8.4±7.6
Glycemia, mg/dl	97.2±11.6	90.2±5.2	96.4±12.5	0.17	94.5±10.6
Total cholesterol, mg/dl	201.9±43.6	198.9±35.5	232.9±39.9	0.04	213.8±41.9
LDL, mg/dl	130.2±38.5	117.8±31.3	143.2±34.0	0.19	131.8±35.2
HDL, mg/dl	55.4±11.9	60.1±22.6	57.6±11.3	0.77	57.8±15.7
Triglycerides, mg/dl	102.3±56.9	89.5±18.5	127.1±75.6	0.21	108.5±59
Creatinine, mg/dl	0.8±0.1	0.8±0.2	0.8±0.1	0.95	.82±0.2

Notes: The table shows the features of the study samples at the beginning of the lifestyle modification protocol. Abbreviations; FM: fat mass; F: visceral Fat; FFM: fat-free mass; TBW: total body water. BMR: Basal Metabolic Rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; MET = Metabolic Equivalent of Task. P values calculated from ANOVA test. Total sample 43 subjects (N). Statistical significance set at $p \leq 0.05$. Data are expressed as mean values ±SD.

Table 2: Mean nutrient intake status of the study participants and % breakdown among meals of the basal diet and differences between groups during the 24-week intervention period

Nutrient	~~Regular~~ pre-menopausal (n=11)	perimenopause (n=14)	~~Menopause~~ post-menopause (n=18)	*p*	Total (n=43)
Calories, Kcal	1538±158	1437±148	1434±146	0.16	1462±153
Carbohydrate, %	53.1±3.4	50.7±3.2	52.5±3.4	0.19	52.1±3.4
Oligosaccharides, g	70.2±8.5	68.6±8.9	74.3±5.7	0.12	71.3±7.9
Starch, g	107.1±16.3	95.2±16.9	96.2±21.2	0.23	98.8±18.8
Total fibre, g	40.6±5.2	41.1±4.2	38.8±3.9	0.33	40.1±4.4
Insoluble fibre, g	30±2.8	30.6±2.3	28.9±2.7	0.44	29,8±2.5
Protein, g	65.0±9.3	63.1±5.9	65.0±9.9	0.79	64.3±8.4
Animal protein, g	18.2±8.0	17.0±3.5	22.7±9.9	0.12	19.5±7.9
Plant protein, g	40.9±5.3	37.4±4.2	36.1±2.5	0.016	37.9±4.3
Animal/Plant protein ratio	0.4±0.2	0.5±0.1	0.6±0.3	0.047	0.5±0.2
Lipids, g	56.1±9.0	53.9±4.3	51.1±5.8	0.14	53.4±6.5
TUFA, g	39.8±7.1	37.3±3.1	33.9±3.3	0.14	36.6±4.8
MUFA, g	29.3±5.3	27.8±2.0	26.1±3.0	0.07	27.5±3.6
PUFA, g	9.6±2.1	9.5±1.7	8.1±1.2	0.03	9.0±1.7
SFA, g	9.9±2.3	9.3±1.0	9.5±1.9	0.72	9.5±1.7
Cholesterol, mg	78.1±28.9	80.4±30.8	88.1±45.7	0.75	82.8±36.3
Alcohol, g	0.0	1.4±5.3	2.1±8.3	0.68	1.3±5.9
% BREAKDOWN AMONG MEALS (calories)					
	Regular (n=11)	Peri menopause (n=14)	Menopause (n=18)	*p*	Total (n=43)
Breakfast, %	18.4±4.2	18.9±1.5	17.6±3.3	0.52	18.3±2.9
Morning Snack, %	10.1±3.9	6.8±3.4	9.8±8.6	0.34	8.7±6.1
Lunch, %	33.1±4.2	32.8±5.5	28.6±6.3	0.09	31.2±5.8

Afternoon Snack, %	9.3±3.2	11.4±3.5	10.0±6.1	0.56	10.4±4.6
Dinner, %	28.1±2.7	29.9±3.7	30.9±9.3	0.62	29.9±6.3
After Dinner, %	6.3±1.9	5.0±2.8	5.7±2.9	0.56	5.6±2.7

Notes: The table shows the average nutrient intake and % distribution between meals of the basal diet and the differences between groups. Abbreviations; TUFA: total unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids. P values calculated from ANOVA test. Total sample 43 subjects (N). Statistical significance set at $p \leq 0.05$. Data are expressed as mean values ±SD.

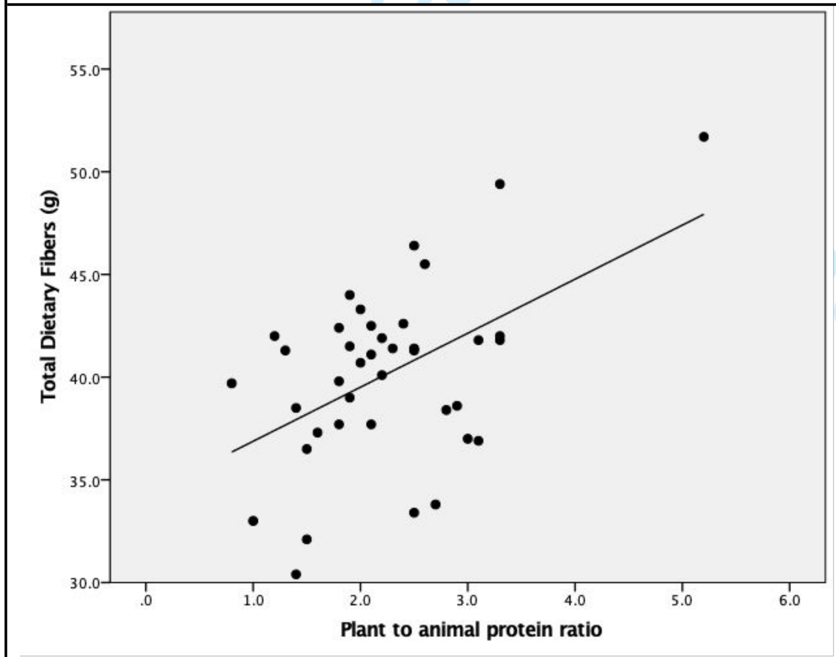
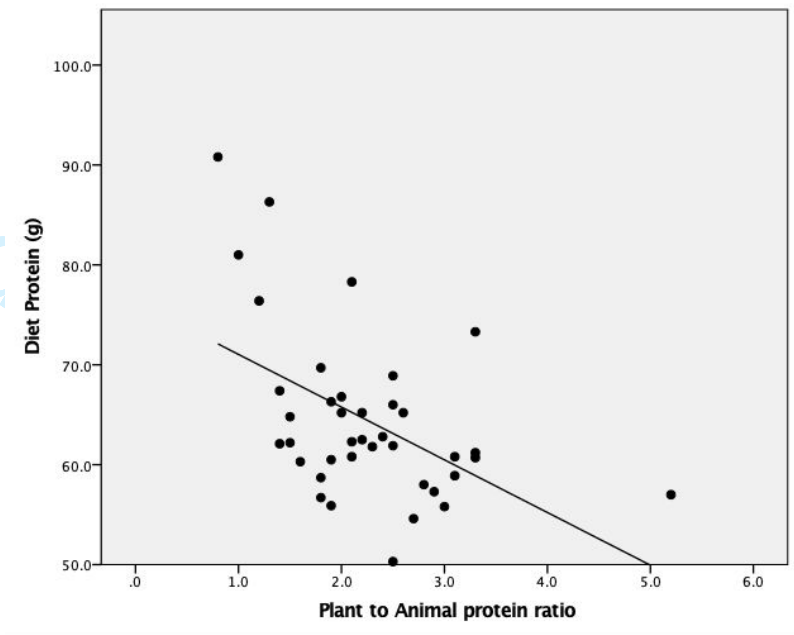
Table 3: Changes in parameters for study subjects between baseline and 8-months follow-up.

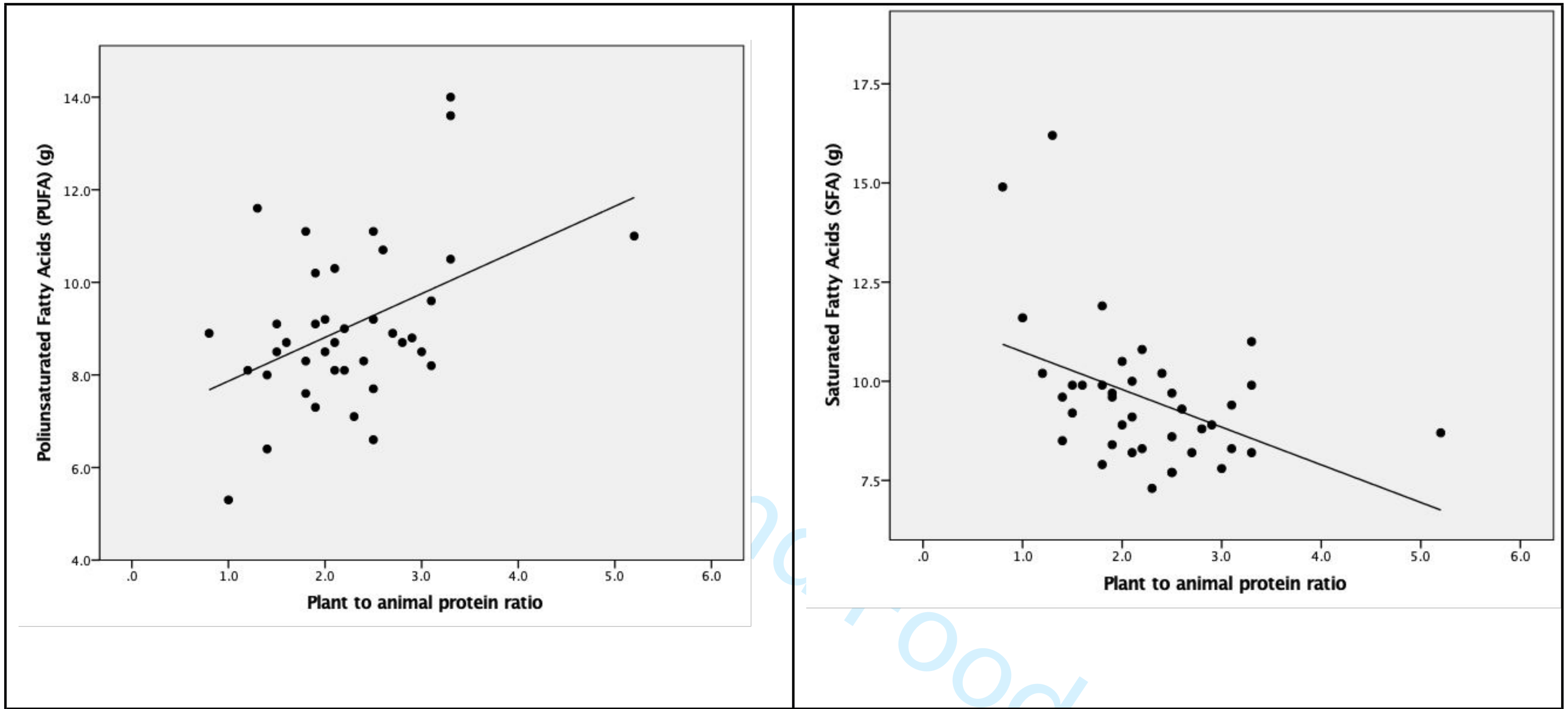
	~~Regular~~ pre-menopausal (n=11)	perimenopause (n=14)	~~Menopause~~ post-menopause (n=18)	*P* *	Total (n=43)	*p* **
∆ Weight, kg	-7.2±4.3	-8.0±4.6	-7.8±5.2	0.92	-7.7±4.7	<0.001
∆ BMI, kg/m^2	-2.8±1.8	-3.0±1.7	-3.0±1.9	0.94	-2.9±1.8	<0.001
∆ FM, kg	-5.1±3.8	-5.7±3.9	-5.8±4.3	0.92	-5.6±4	<0.001
∆ FFM, kg	-1.7±1.3	-2.2±1.3	-1.9±1.5	0.61	-2.0±1.4	<0.001
∆ TBW, kg	-1.5±1	-2.0±1.1	-1.6±1.4	0.62	-1.7±1.2	<0.001
∆ Body Protein, kg	-.2±0.4	-0.3±0.3	-0.3±0.6	0.78	-0.3±0.5	<0.001
∆ Waist circumference, cm	-4.8±2.9	-3.6±3.8	-4.0±2.8	0.66	-4.1±3.1	<0.001
∆ Hip circumference, cm	-8.0±5.4	-5.4±3.2	-5.2±4.1	0.19	-6.0±4.3	<0.001
∆ BMR, kcal	-72.7±49.5	-80.6±47.1	-65.6±44.9	0.67	-72.3±46.1	<0.001
∆ Systolic BP, mmHg	-4.5±16.5	-10.6±10	-5.8±15.2	0.50	-7.0±14	0.002
∆ Diastolic BP, mmHg	-4.8±13.8	-2.2±5.6	-3.4±8	0.78	-3.4±9	0.02
∆ Fasting blood glucose, mg/dl	-5.6±8.7	-0.3±12.4	-1.6±8.6	0.5	-2.1±9.9	0.22
∆ Triglycerides, mg/dl	-6.3±35	-7.5±28.9	-27.5±62.6	0.47	-16.0±47.8	0.06
∆ Total cholesterol, mg/dl	-10.8±15.3	-10.3±30.1	-28.8±46.1	0.35	-18.6±36.2	0.005

Δ HDL-cholesterol, mg/dl	4.0±3.2	-1.5±11.7	0.2±10.4	0.47	0.6±9.7	0.004
Δ LDL-cholesterol, mg/dl	-13.8±13.5	-6.5±10.7	-19.3±36.2	0.48	-13.8±25.7	0.74
Δ Creatinine, mg/dl	0.03±0.1	-0.02±0.12	0.03±0.15	0.64	0.01±0.13	0.5

Notes: The table shows the changes in anthropometric characteristics and blood parameters between baseline and 8-month follow-up in the study groups. Abbreviations; FM: fat mass; FFM: fat-free mass; TBW: total body water; BMR: Basal Metabolic Rate. P values calculated from ANOVA test. Total sample 43 subjects (N). Statistical significance set at $p \leq 0.05$. Data are expressed as mean values ±SD. *between groups **total sample

Figure 1 - Correlation of plant to animal protein ratio with nutritional and blood parameters in the whole study sample.

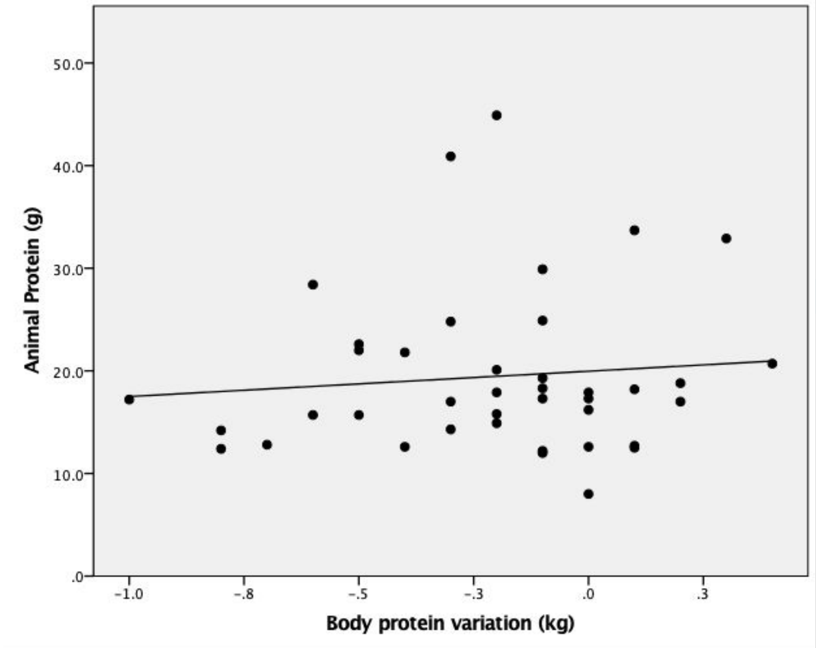
<table>
<tr><td>A - Total dietary fibre vs. plant to animal protein ratio (r = 0.495; p = 0.001)</td><td>B - Dietary protein vs. plant to animal protein ratio (r = -0.503; p = 0.001)</td></tr>
<tr><td>
</td><td>
</td></tr>
<tr><td>C - PUFA vs. plant to animal protein ratio (r = 0.438; p = 0.005)</td><td>D - SFA vs. plant to animal protein ratio (r = -0.434; p = 0.006)</td></tr>
</table>



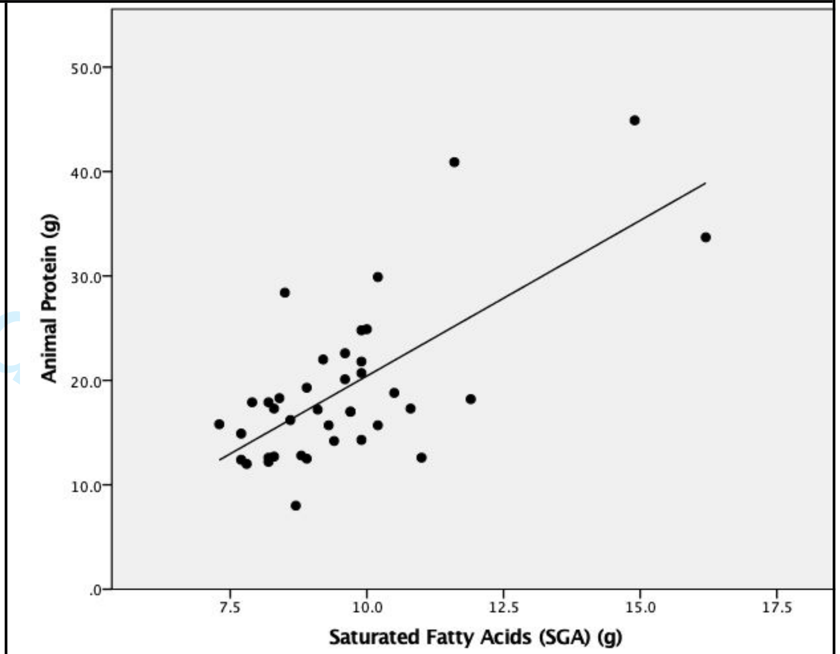
Notes: The figure shows the correlation between plant/protein ratio and dietary fiber (A), total dietary protein (B), PUFA (C), and SFA (D) in the entire study sample after the lifestyle intervention. Differences in proportions of discrete traits were investigated using Pearson's correlation test. Total sample 43 subjects (N). Statistical significance set at $p \leq 0.05$. PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids.

Figure 2 - Correlation between the type of protein in the diet and some nutritional and blood parameters in the entire study sample.

A - Animal protein in the diet vs. body protein variation (r = 0.14; p = 0.14)

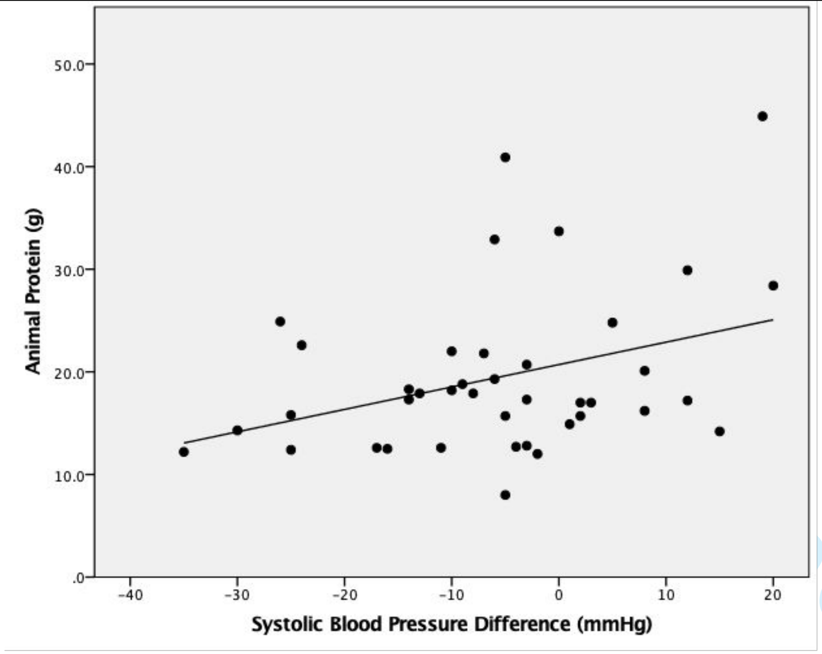


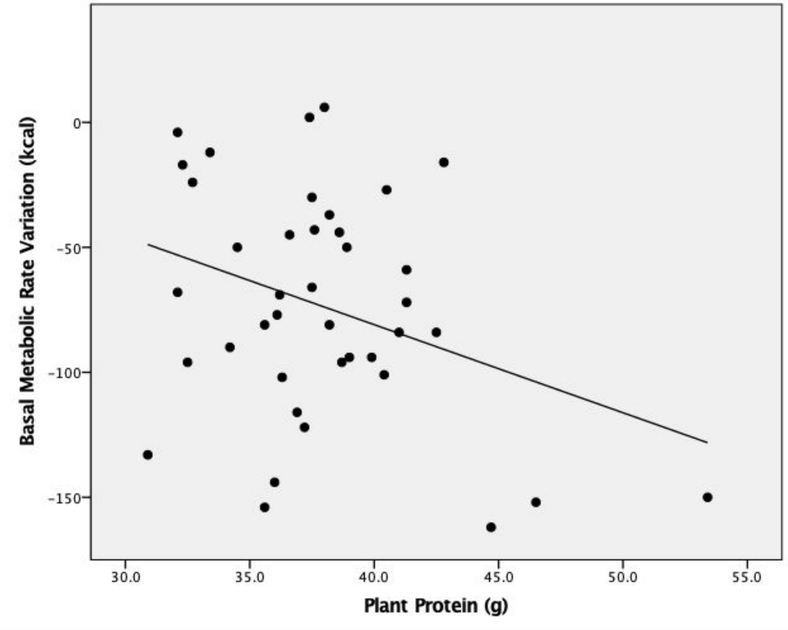
B - Animal protein in the diet (g) vs. SFA (r = 0.689; p < 0.001)



C - Animal protein in the diet (g) vs. systolic blood pressure difference (r = 0.358; p = 0.023)

D - Variation in BMR vs. plant protein (r = -0.334; p = 0.035)



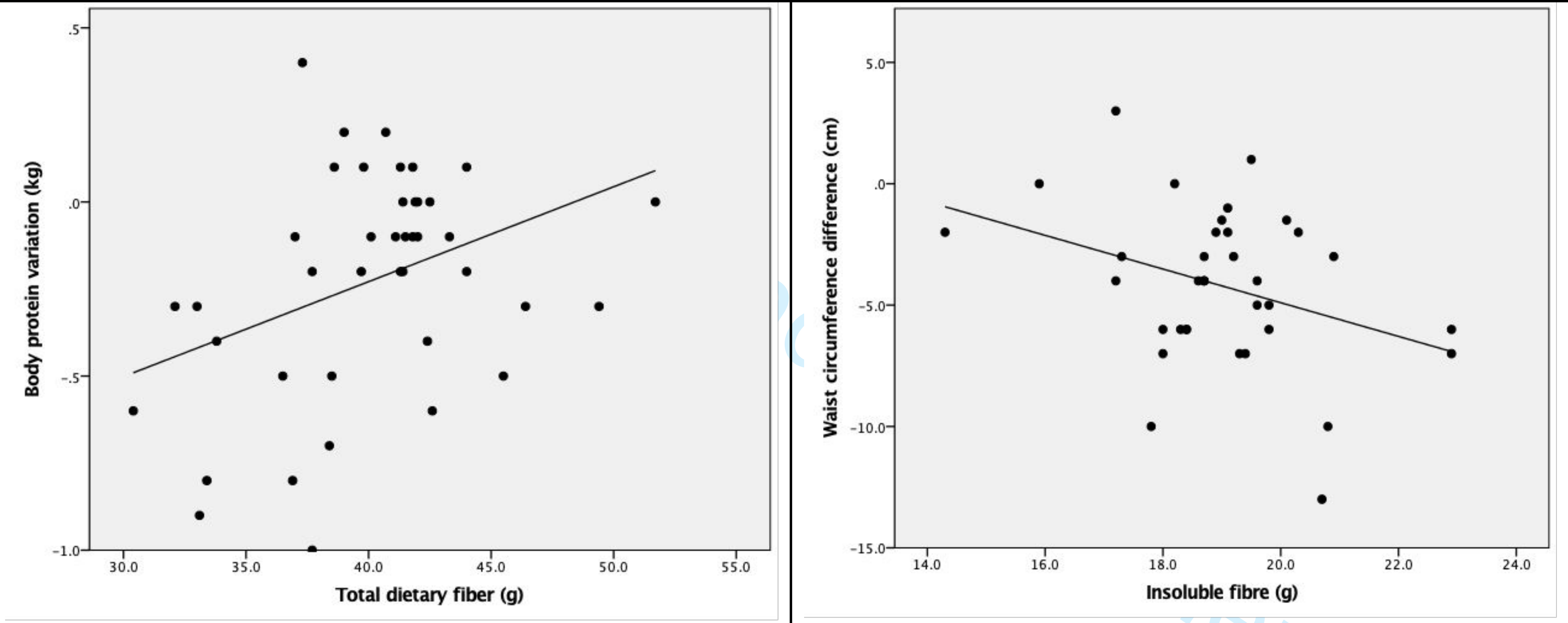


Notes: The figure shows the correlation between daily animal protein in the diet and body protein variation (A), saturated fatty acids (SFA) (B), and systolic blood pressure difference (C). Relationship between the variation in BMR and plant protein in the diet after the lifestyle intervention is also shown (D). Differences in proportions of discrete traits were investigated using Pearson's correlation test. Total sample 43 subjects (N). Statistical significance set at $p \leq 0.05$.

Figure 3 - Correlation between dietary fiber content and body composition parameters

A - Body protein variation vs. total dietary fibre (r = 0.438; p = 0.004)

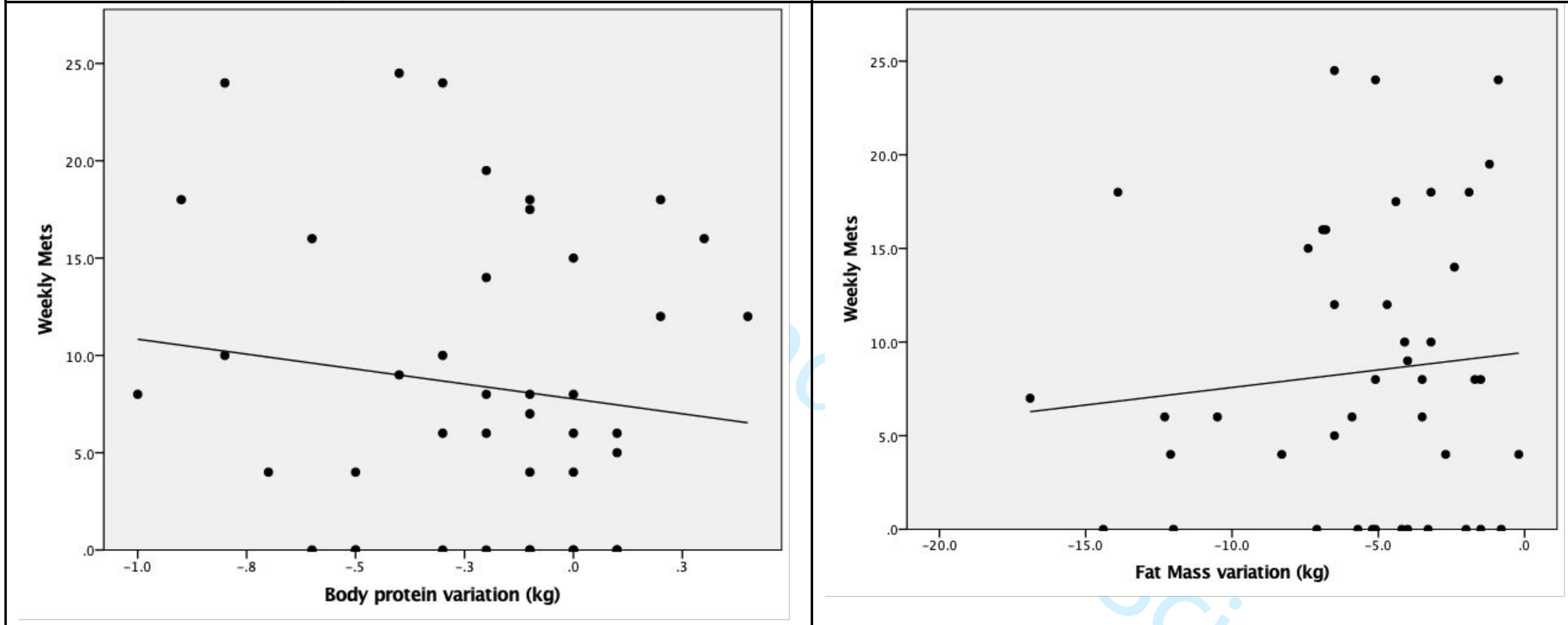
B - Waist circumference difference vs. insoluble fibre in the diet (r = -0.351; p = 0.036)



Notes: The figure shows the correlation between body protein variation and total dietary fibre (A) and waist circumference variation and insoluble fibre (B) after lifestyle intervention. Differences in proportions of discrete traits were investigated using Pearson's correlation test. Total sample 43 subjects (N). Statistical significance set at $p \leq 0.05$.

Figure 4 - Correlation between average weekly physical activity expressed in METs and body composition parameters.

A - Weekly METs vs. body protein variation (r = -0.309; p = 0.043)	B - Weekly METs vs. fat mass variation (r = 0.01; p = 0.99)



Notes: The figure shows the correlation between weekly METs and body protein (A) and FM (B) variation in all subjects after the lifestyle intervention. Differences in proportions of discrete traits were investigated using Pearson's correlation test. Total sample 43 subjects (N). Statistical significance set at p ≤ 0.05. MET = Metabolic Equivalent of Task