

Research Article

Human Ration, as Part of a Dietary Program for Weight Loss, Improves Iron Status, Dietary Fibre Intake and Does Not Impair Zinc Metabolism and Bone Health in Overweight Women

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Abstract

We evaluated whether Human Ration (HR), as part of a dietary energy restriction, may play a role in body weight control, dietary intake, body composition, bone health and blood pressure more than dietary energy restriction alone. Free-living, overweight and obese women (n=76, Body Mass Index (BMI) 25 to 45; aged 24-45) were randomized to receive one serving per day of a beverage containing HR (20g) or a placebo beverage (control), as part of a reduced energy (15% of Energy Estimated Requirements deficit) dietary program. HR beverage increased the dietary fibre consumption and decreased the intake of carbohydrate and total fat. HR provided

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an increase in haemoglobin concentration (P=0.0001) and did not impair zinc absorption. Weight loss was not different between groups (P=0.924) but waist circumference decreased in HR treatment (P<0.01). No differences were observed in bone densitometry, biomarkers or blood pressure. A positive association was observed for bone mineral density and body mass index (r=0.3956, P=0.0127) and a negative one between erythrocyte zinc and BMI (r=-0.5123; P=0.0005). HR did not potentiate weight loss as part of a dietary energy restriction program, but ameliorated the diet quality and it was effective in improving iron status, without impairing zinc metabolism.

Keywords: Body composition; Bone health; Dietary fibre; Dietary supplements; Mineral status; Whole grains

Introduction

Overweight has been considered a protective factor against bone loss, osteoporosis and fractures [1]. However, recent publications indicate that this relation is controversial, since the accumulation of fat mass may have a negative on bone health [2]. The subclinical inflammation induced by obesity is followed by significant increases in levels of proinflammatory cytokine and the inflammatory scenario increases bone turnover and reabsorption markers [3]. Despite weight reduction being a strategy of inflammation control, rapid weight loss (10% of body weight in less than six months) is associated with bone loss [4,5].

An optimum weight loss comes from the induction of a negative energy balance, associated with high-quality nutritional food [6]. Regular consumption of dietary fibre is a part of these strategies, since their consumption is associated with lower energy intake [7], either by adopting a healthy eating pattern, with increased intake of fruits and vegetables [8] or by the dietary fibre supplements intake [9].

The effect of dietary fibre in the lower energy intake is based on the early satiation and maintenance of a longer satiety. Such effects are the key points of the pathway that involves the physiological effects of dietary fibre on body weight [10].

Based on evidence demonstrating the positive effect of dietary fibre intake on weight control [11-13], products rich in dietary fibre have been developed with the purpose of reducing bodyweight, such as mixture of food ingredients recognized by its high content of dietary fibre, vitamins and minerals. In Brazil, a mix of cereals, grains and seeds, known as "Human Ration" (HR), has been widely marketed in the last decade, with the proposal to assist in weight loss. The product is composed of several food ingredients, including oat bran, sesame, flaxseed and wheat fibre, which alone have a positive effect on human health [14-16]. However, previous clinical trial showed that weight loss was not altered by HR intake, despite it improved lipid profile [17].

Regarding the great diversity of ingredients in HR, this mixture is considered rich in micronutrients, such as iron, zinc, magnesium, B complex vitamins, as well as bioactive compounds, unsaturated lipids

and dietary fibre. All of these components have been associated with potential health positive effects, such as reduction on development risk of obesity, diabetes, hypertension and cardiovascular disorders. Therefore, this study aimed to assess the effect of HR intake, as part of a dietary program for weight loss, on nutrient intake, weight loss, body composition, blood pressure, bone health, iron and zinc status in overweight women.

Methods

Subjects

Potential participants were recruited through a database of the public health service using the following criteria: female gender, body mass index (BMI) 25-35kg/m², aged 24-45 years. The recruited subjects were screened with interviews when the researchers measured weight and height to calculate BMI. To complete the eligibility, the subject should drink milk at breakfast and presented no intention of changing the physical activity pattern during the study period. Exclusion criteria included: pregnancy, lactation, postmenopausal or ovariectomized women, intolerance or allergy to any ingredient of HR, use of some other strategy for weight loss (drugs, herbal or nutritional supplements), use of anti-inflammatory drugs or medicines that interferes with bone mineral metabolism, presence of renal or gastrointestinal disease, history of endocrine disease, fractures in the past six months and/or immobilization for more than two months. Also excluded were those that presented bodyweight fluctuations greater than 5% in in the past two months, according to Maki et al. [18]. The research was approved by the Federal University of Viçosa Ethics Committee on Human Research, Viçosa-MG, Brazil (Ref. No. 009/2011) and registered in the Brazilian Registry of Clinical Trials (Trial RBR-4QPJ2R).

Study design

This was a prospective, randomized, single blind crossover (2x2) study. The trial included a 1-week screening/baseline period followed by 5 weeks of each treatment. After the first period (A) there was a 1-week washout followed by another 5-week period (B). Two groups of volunteers were randomly formed, G1 (n=11) and G2 (n=11). In each period, two treatments were applied randomly: 15% energy restriction associated with placebo drink consumption (control treatment, not containing HR); or 15% energy restriction associated with the HR drink (HR treatment, containing HR). G1 underwent the control treatment during period A, whereas G2 underwent HR treatment. After 1-week washout, in period B, there was a reversal in the order of the treatments, as shown in figure 1.

During the baseline period, all subjects were evaluated: anthropometry, body composition, nutrient intake, blood pressure, physical activity, serum biochemical markers of bone health and serum biochemical markers of iron and zinc status. All these parameters were reassessed at the end of both treatment periods, beyond the end of the washout period. During periods A and B, anthropometric parameters, food intake, blood pressure and physical activity were evaluated every 2 weeks.

Preparation and composition of study product

For HR preparation, the following ingredients were used: wheat fibre (20.41%), defatted soy flour and tritirated flaxseed (10.20%), brown rice flour, rolled oats, white corn meal, tritirated sesame white

and brown sugar (8.16% each), wheat germ (6.12%), agar-agar (unflavoured gelatin; 4.08%), quinoa, cocoa powder, guarana powder and beer yeast (2.04% each). HR supply to the subjects was carried out in a drink based on milk containing: HR (20g), defatted milk powder (15g), 50% cocoa chocolate powder (7g) or strawberry flavour (10g) and xanthan gum (0.3g). The placebo drinks had the same components, except HR. The amount of water (150mL) to perform the dilution was measured under a standardized measuring cup.

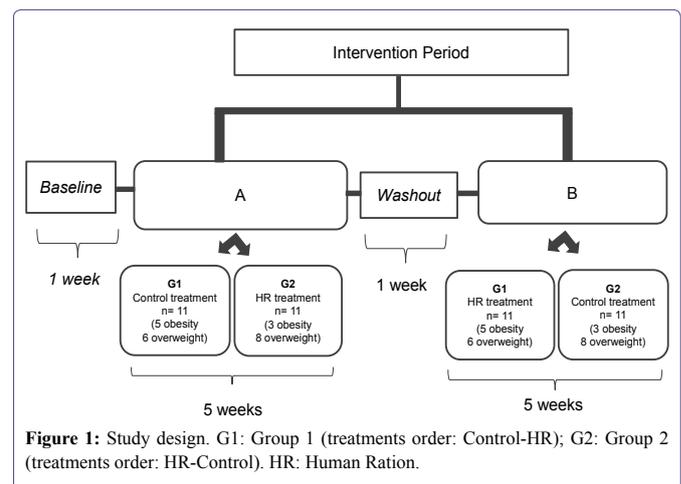


Figure 1: Study design. G1: Group 1 (treatments order: Control-HR); G2: Group 2 (treatments order: HR-Control). HR: Human Ration.

The drink's chemical composition was carried out according to the Association of Official Analytical Chemistry [19]. The mineral content was quantified by absorption spectrophotometry in plasma [20]. The vitamin content was determined by HPLC using the chromatographic conditions proposed by Guinaz et al. [21].

Measurements

Diet and nutrient intake

The dietary energy restriction was based on 15% of Estimated Energy Requirement (EER) and the dietary weight-loss program was planned by nutritionist researchers, with a normal distribution of macro- and micronutrients [22].

At weeks 0, 5, 6, 7 and 12, all subjects completed 3-day food records. The researchers instructed the subjects about the correct register using visual tools with suitable procedures to ensure the accuracy. Dietary intake data were analysed using Dietpro Software version 5.1i, (Nutrition and Health Department, Federal University of Viçosa, Brazil). All data entry was performed twice by two different researchers. Nutritional information about reported foods that were not in the software database, were obtained in other food composition tables and the data was added to the software database. Nutrient information was also obtained through food labels or recipes from subjects.

Physical activity level

The usual level of physical activity was assessed by the International Physical Activity Questionnaire (IPAQ) version 6, proposed by WHO and validated for Brazilians by Pardini et al. [23]. This evaluation was performed at weeks 0, 5, 6, 7 and 12.

Anthropometry, body composition, bone densitometry and blood pressure

Body weight and height were measured according to WHO recommendations [24]. BMI was calculated from height and weight data. Waist circumference was measured at the umbilicus. For classification we used WHO references and values greater than 80 cm were considered inadequate abdominal adiposity [25].

Body composition and Bone Mineral Density (BMD) were evaluated by DXA; GE HealthCare, Lunar Prodigy Advance®) and specialized software (Encore® version 13:31), based on a previously validated procedure. DXA performance was checked daily, including measurement of the manufacturer's phantom. To conduct the evaluation, it was required that the volunteers were barefoot, using only an apron without any metallic material, presenting while still fasting and the bladder emptied. The menstrual cycle of the volunteers was observed, in order to avoid this period. Fat ratio higher than 32% was considered as risk according to Lohman [26]. For BMD evaluation, the Z score was calculated and compared to individuals of the same age, sex, weight and ethnicity. Normal values were considered those who were up to the limit of -1 standard deviation, calculated by reference to the mean bone-mineral density peak in young adults, according to the Brazilian Consensus on Osteoporosis [27].

Resting blood pressure was assessed after the participant had been seated for at least 5 minutes. Participants refrained from ingesting caffeine during the 30 minutes preceding the measurement. Blood pressure was measured using an automatic blood pressure device (G. Tech, Master Line®). Two measurements were taken, separated by at least 2 minutes and averaged.

Laboratory measurements

Fasting venous blood samples (10mL) were collected at baseline, 5, 7 and 12 weeks. Bone-Specific Alkaline Phosphatase (BALP) was measured by chemiluminescence (Modular, Roche Diagnostics®). Parathormone and C-telopeptide of type I collagen (CTx) concentrations in plasma were measured by an electrochemoluminescence method (Modular, Roche Diagnostics®). Serum phosphorus and calcium and ionic calcium were measured by colorimetric methods using UV kits (Bioclin®) in an automatic analyser for clinical chemistry (BS 200; Mindray®), calibrated with reagent for analytical tests in automated methodologies and patterns of normal and pathological levels. Iron nutritional status was accompanied by dosage of a complete haemogram using an electrical impedance method (Coulter T890; Beckman Coulter®) and ferritin by chemiluminescence (Centaur; Siemens®). Plasmatic and erythrocyte zinc evaluated by means of atomic absorption spectrometry (GBC 908 AA spectrophotometer®) [28].

Statistical analysis

The sample size calculation was performed according to the method proposed by Mera, Thompson and Prasad [29], adopting a statistical power of 80%. The percentage of body fat was considered as a dependent variable. Results are reported as mean±SD. Skewness/Kurtosis tests were used to determine variable distribution. Descriptive statistics were used to describe demographic characteristics of the study population. Chemical compositions of the study drinks were

analysed by Turkey test. To analyse differences from treatment means and change means on anthropometric and laboratory measurements, Student's test or Mann Whitney U-test was used. To detect significant differences in dietary intake before and after each intervention, the paired *t*-test or its corresponding non-parametric Wilcoxon matched-pairs test were used. Pearson or Spearman correlation was used to analyse association between anthropometric and plasma markers, according to data distribution (Table 1).

Results

Participant characteristics

Seventy-six volunteers were screened and 32 met the inclusion criteria. Twenty-seven began the study and 24 finished all the phases (Figure 2). The average age of participants was 34.5 years. Body-weight and BMI mean were 73.84±9.53kg and 29.07±2.87kg/m², respectively. According to BMI, 63.63% of members were overweight and 36.37% were obese. The volunteers presented as average schooling, 7.45±3.64 years of study. 91% presented low family income. 22.72% of the volunteers were nulliparous, 63.63% had between 1 and 2 children and 13.63% had three or more children. 46% were classified as low active and the physical pattern did not change during the study.

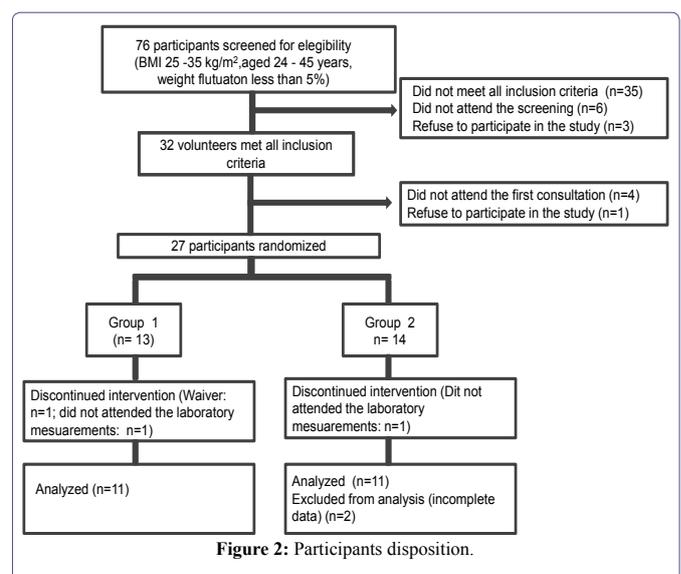


Figure 2: Participants disposition.

Drinks chemical composition

Each serving of HR drink presented 4.06 and 4.17 g of total dietary fibre for chocolate and strawberry flavours, respectively ($P>0.05$). The drinks had on average 16.5% of daily recommended total fibre Adequate Intake (AI) and 96% of phosphorus Daily Recommended Intake (DRI). One HR drink serving had 30% of calcium DRI. Iron content of the HR drinks was higher than that of placebo drinks ($P<0.05$).

Dietary intake

Reductions in total fat intake and monounsaturated and polyunsaturated fat were equally seen in both interventions ($P<0.05$). Intake of saturated fat decreased only in the control treatment ($P=0.029$).

Cholesterol intake did not change in any of the groups and remained within the standard reference intake. HR treatment decreased carbohydrate intake ($P=0.013$) and control treatment decreased protein intake ($P=0.026$). Manganese intake was higher after HR treatment. Total dietary fibre intake did not change during any of the interventions,

but HR treatment presented a higher consumption at baseline (31%) and endpoint (28%) than the control group. Calcium intake was inadequate throughout the study in both groups. Sodium intake remained within recommended levels for the control treatment, but HR treatment showed a higher intake just above DRI level (Table 2).

Composition	Control Drink Strawberry	Control Drink Chocolate	HR Drink Strawberry	HR Drink Chocolate
g•100g ⁻¹				
Moisture	4.72±0.27 ^A	4.71±0.27 ^B	6.67±0.49 ^A	6.4±0.51 ^A
Ash	5.31±0.15 ^A	5.49±0.05 ^B	4.11±0.05 ^B	4.23±0.03 ^B
Protein	19.66±0.55 ^A	21.82±0.43 ^A	19.89±0.00 ^A	20.62±0.84 ^A
Lipid	4.83±0.43 ^B	6.24±0.44 ^B	13.24±1.38 ^A	11.42±0.22 ^A
Total dietary fiber	4.08±0.49 ^B	4.63±0.45 ^B	9.26±0.53 ^A	9.66±1.87 ^A
Soluble dietary fiber	3.15±0.13 ^{AB}	2.07±0.33 ^B	2.12±0.38 ^A	1.93±0.21 ^{AB}
Insoluble dietary fiber	0.95±0.62 ^B	2.59±0.78 ^B	7.16±0.91 ^A	7.74±2.09 ^A
Carbohydrate	65.1±1.11 ^A	55.8±1.11 ^B	45.21±1.12 ^C	42.19±0.85 ^C
Energy (kcal)	379.41±1.67 ^{AB}	366.76±1.23 ^B	382.64±11.61 ^A	354.13±2.31 ^C
mg•100g ⁻¹				
Iron	2.84±0.37 ^B	3.10±0.42 ^B	4.23±0.07 ^A	3.78±0.19 ^A
Zinc	2.24±0.21 ^D	2.83±0.09 ^C	3.49±0.04 ^B	4.38±0.17 ^A
Calcium	899.0±61.0 ^A	576.3±8.83 ^B	619.9±17.6 ^B	498.8±1.28 ^C
Sodium	6.35±0.15 ^A	4.57±0.08 ^B	3.95±0.08 ^C	3.95±0.08 ^C
Manganese	0.00±0.00 ^B	0.00±0.00 ^B	2.82±0.01 ^A	3.08±0.23 ^A
Magnesium	93.3±7.3 ^C	143.3±3.98 ^B	155.2±2.61 ^B	181.3±6.35 ^A
Potassium	10.47±1.98 ^B	11.07±0.79 ^B	17.06±1.13 ^A	14.85±1.77 ^{BB}
Phosphorus	681.58±65.84 ^A	661.10±23.51 ^A	670.38±21.54 ^A	721.77±25.81 ^A
Copper	0.00±0.00 ^C	0.62±0.01 ^A	0.4±0.02 ^B	0.68±0.007 ^A
Vitamin E	ND	ND	3.96	3.96

Table 1: Chemical composition of drinks (dry samples).

Note: Results are expressed as mean±SD from three repetitions.

Means followed by the same small letter in the lines, are not significantly different at 5% probability by Turkey test.

HR: Human Ration, ND: Not determined.

Variable	Control Treatment			HR Treatment			P Value Between Changes
	Baseline	Endpoint	P	Baseline	Endpoint	P	
Energy (kcal/day)	1323±327.91	1361.36±380.61	0.543	1259.20±322.82	1268.85±260.60	0.681	0.886
Carbohydrate (% energy)	52.19±8.35	48.37±8.11	0.147	53.37 ± 9.54	45.59±8.20	0.013*	0.927
Protein (% energy)	14.42±3.94	12.26±2.41	0.026*	14.54 ± 3.29	12.50±3.41	0.144	0.261
Total Fat (% energy)	33.39±6.99	25.95±4.96	0.002*	34.12±5.74	27.79±5.27	0.001*	0.879
Monounsaturated Fat (% energy)	9.43±1.79	7.73±2.02	0.012*	10.40±2.62	7.89±2.22	0.009*	0.715
Polyunsaturated Fat (% energy)	10.17±3.13	7.89±1.75	0.048*	10.94±2.46	8.48±2.44	0.008*	0.447
Saturated Fat (% energy)	11.50±7.59	7.94±2.10	0.029*	8.88±3.47	7.69±2.14	0.184	0.827
Cholesterol (mg/d)	222.11±201.84	175.90±96.73	0.546	187.99±114.78	152.82±75.95	0.349	0.579
Dietary fiber (g/d)	14.53±6.49	16.41±6.67	0.169	21.07±5.39	22.79±6.62	0.327	0.605
Calcium (mg/d)	506.50±128.50	561.47±125.23	0.149	490.72±203.96	631.76±275.28	0.952	0.784
Magnesium (mg/d)	240.33±166.21	216.56±68.64	0.778	209.12±55.58	228.74±60.39	0.17	0.851
Sodium (mg/d)	1987.97±733.03	1935.83±1047.41	0.498	2517.84±850.17	2446.69±759.18	0.327	0.309
Iron (mg/d)	7.53±4.17	6.38±1.65	0.314	6.59±2.35	6.78±2.04	0.777	0.903
Zinc (mg/d)	8.33±4.26	7.37±1.62	0.205	7.84±3.15	7.51±2.29	0.931	0.338

Table 2: Dietary intake during the study.

Note: Data are mean±standard deviation. Values followed by * are statistically significant. $P<0.05$ from paired t test or Wilcoxon signed rank test.

HR: Human Ration.

Anthropometry, body composition, bone health, iron and zinc status and blood pressure

Both treatments resulted in weight loss, without differences between them (P=0.92). Weight loss ranged from 0.16 to 4.66% of body weight from baseline to endpoint. Waist circumference decreased in the HR treatment (-2.54±0.58 vs. -1.12±0.58cm, P<0.01). Body fat was not modified by treatments (P>0.05).

Blood pressure values presented within normal parameters (blood pressure less than 130/85mmHg). There were no differences in systolic (P=0.121) and diastolic (P=0.283) levels when comparing the treatments, but at the endpoint of the control treatment we observed an increase in SBP (P=0.036; Table 3).

Haemoglobin concentration increased in HR treatment (P=0.0003) and it was significant when compared to control (P=0.0001; Table 3). Zinc status did not change. However, erythrocyte zinc concentration and BMI had a negative association (r=-0.5123; P=0.0005), independent of treatments.

DXA analysis did not demonstrate any change for bone mass and bone mineral density. Bone turnover biomarkers (CTX and BALP) also did not change during the intervention period. Plasma levels of PTH, calcium and phosphorus did not change during the intervention period (Figure 3). It is noteworthy that a positive association between BMI and BDM was observed (r=0.3749; P=0.0122).

Discussion

In women, schooling is negatively associated with BMI and presents itself as the main socioeconomic factor associated with obesity [30,31]. We found these characteristics in the study subjects. Obesity has grown more pronounced among poor populations and women that come from higher-income families were less likely to develop overweight [32,33].

The drinks used in this study present a calcium content enough to provide 30% of the mineral DRI. The large concentration of calcium

was due to milk as the drinks' basis. HR itself cannot be considered an important source of calcium. HR drinks contain high iron concentration average (4.00mg·100g⁻¹) than placebo drinks. HR drinks also contain antioxidant micronutrients such as zinc and vitamin E. These micronutrients act in oxidative damage prevention by operation with enzymatic complexes (catalase, peroxidase and superoxide dismutase), by stabilizing membranes and by suppressing reactive oxygen species [34,35].

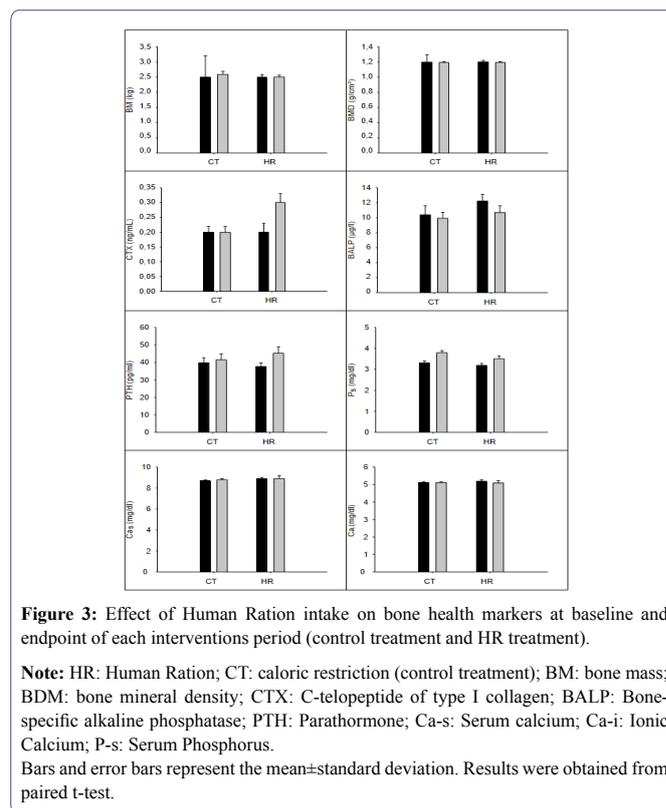


Figure 3: Effect of Human Ration intake on bone health markers at baseline and endpoint of each interventions period (control treatment and HR treatment).

Note: HR: Human Ration; CT: caloric restriction (control treatment); BM: bone mass; BDM: bone mineral density; CTX: C-telopeptide of type I collagen; BALP: Bone-specific alkaline phosphatase; PTH: Parathormone; Ca-s: Serum calcium; Ca-i: Ionic Calcium; P-s: Serum Phosphorus.

Bars and error bars represent the mean±standard deviation. Results were obtained from paired t-test.

Variables	Control Group		P ⁽¹⁾	HR Group		P ⁽¹⁾	P ⁽²⁾	P ⁽³⁾
	Baseline	Endpoint		Baseline	Endpoint			
Weight (kg)	72.8±2.0 (64.1;80.3)	72.1±2.1 (63.3;81.6)	0.014	72.9±2.0 (64.4;81.2)	72.1±2.1 (63.5;80.9)	0.029	0.924	0.962
BMI (kg/m ²)	28.8±0.6 (26.3;31.2)	28.4±0.6 (26.0;31.1)	0.009	28.8±0.6 (25.9-31.2)	28.5±0.6 (25.8;30.87)	0.027	0.759	0.860
WC (cm)	96.0±1.6 (90;102.4)	94.9±1.7 (88.5;106.1)	0.794	97.1±1.6 (91.4;103.5)	94.6±1.6 (89;102.2)	0.0003	0.001	0.631
SBP (mmHg)	120.2±4.5 (110;120)	126.4±4.5 (115;132)	0.036	126.9±5.3 (111;134)	124.5±5.5 (108;136)	0.672	0.121	0.162
DBP (mmHg)	78.5±2.8 (73;80)	75.1±4.1 (68;84)	0.591	73.7±2.3 (67;80)	74.5±2.5 (66;79)	0.845	0.283	0.142
BF (%)	39.9±0.9 (37.7;44.2)	39.8±0.9 (38;43.4)	0.362	40.5±0.9 (37.1;44.2)	39.8±0.9 (37.7;43.6)	0.057	0.196	0.672
LM (%)	56.6±0.8 (52.8;59.4)	56.59±0.9 (53.3;58.4)	0.909	56.0±0.9 (52.7;59.5)	56.6±0.9 (53.3;59.2)	0.083	0.171	0.634
Hb (g/dL)	12.91±0.4 (12.9; 14.0)	12.93±0.21 (12.6; 13.6)	0.108	12.71±0.33 (12.4; 13.5)	13.26±0.31 (12.7; 14.1)	0.0003	0.0001	0.246
Ferritin (ng/mL)	72.51±48.6 (33; 92.3)	71.26±55.2 (30; 109.55)	0.027	69.83±57.3 (28.5; 93.5)	68.75±54.2 (33.5; 78.6)	0.664	0.2153	0.860
EZn (µgZn/g Hb)	35.03±1.4 (31.2; 38.3)	31.57±2.04 (24.2; 39.0)	0.987	30.72±2.02 (25.5; 33.5)	30.37±1.21 (26.7; 34.1)	0.834	0.336	0.088
PZn (µg/mL)	71.84±6.5 (55.0; 94.5)	63.63±1.27 (59.0; 66.7)	0.332	69.63±4.39 (60.0; 75.0)	62.27±1.52 (56.0; 67.65)	0.098	0.859	0.484

Table 3: Change in anthropometric, metabolic and clinical variables after five weeks treatment.

Note: BMI: Body Mass Index; WC: Waist Circumference; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; BF: Body Fat; LM: Lean Mass; Hb: Hemoglobin; EZn: Erythrocyte Zinc; PZn: Plamatic Zinc; HR: Human Ration.

Data are mean±standard deviation (95% confidence interval).

(1) P<0.05 from paired t-test or for Wilcoxon matched-pairs signed rank test, as statistical within group differences (baseline vs. endpoint).

(2) P<0.05 from Student's t-test or for Mann Whitney test, as statistical significance between diet differences (Control vs. HR).

(3) P<0.05 from paired t-test or for Wilcoxon matched-pairs signed rank test, effect of washout (comparing the baselines).

The dietary fibre content in HR drinks was higher than control drinks, since HR is composed of many cereal, grains and whole seeds. In contrast to the present study, Melanson et al. [13], observed an increase in dietary fibre intake when evaluating the effect of a hypocaloric diet associated with the consumption of whole grains. Maki et al. [18], states that the lower consumption of total dietary fibre appears to be attributable to energy intake, but in the present study we did not observe this association.

In our research, both interventions were based on hypocaloric diets differentiated by the consumption or not of drinks containing cereals, grains and whole seeds. In the control treatment, mean of total dietary fibre intake was 11.52g/1,000kcal. HR treatment showed higher consumption: 17.34g/1,000kcal. Our initial hypothesis was based on the statement that the consumption of a product such as HR increases dietary fibre intake. Nutritional guidance and hypocaloric diet was not able to reach the adequate total dietary fibre intake in control treatment. However, HR was able to maintain a high fibre intake during the intervention period (Table 2). Consumption of fruits, vegetables, cereals, grains and whole seeds as a habit has been associated with lower occurrence of overweight due to higher dietary fibre and micronutrients intake [36,37]. In our study the increased total fibre intake in HR treatment supports this hypothesis as well as the greatest reduction of waist circumference in this treatment (Table 3).

The hypocaloric nutritional guideline associated with HR did not potentiate weight loss when compared with control treatment. Marques Rocha et al. [17], also did not observe a considerable weight loss using a similar HR drink.

A high level of dietary fibre food is associated with reductions in waist circumference [38,39]. We observed a reduction in waist circumference during HR consumption, which may suggest a change in the fat distribution pattern. Rocha et al. [17], did not observe a waist circumference change in women who consumed HR. However, Maki et al. [18], observed a greater reduction in waist circumference in treatment with higher fibre content even though no differences were observed in bodyweight between groups. The related mechanisms involve hormonal and colonic factors that contribute to a greater feeling of satiety and satiety reducing food intake, increased lipolysis and reduction of lipid deposition [10].

Our weight-loss program was based on a hypocaloric diet designed to ensure a negative energy balance. However, the level of caloric restriction may not have been sufficient to produce the expected results on bodyweight control. Subjects were instructed to follow the proposed dietary plan and to record food intake in for 72h. We did not observe a difference between the caloric prescribed and the caloric intake (data not shown), but underestimation of energy consumption is a common practice among overweight people and consequently foods that are not part of a healthy diet may be omitted [40-42].

The parameters that make up bone health did not change. For HR treatment, a reduction in bone mass content close to the statistical significance level was observed ($P=0.066$), which may cause concern. However it was not accompanied by changes in CTX bone-resorption marker. The lack of significant variation in CTX indicates that weight loss did not cause damage to bone health, since DXA measurements are not able to acknowledge acute changes [43].

The association between bone mineral density and BMI was found in this study, as demonstrated by Glover et al. [44]. The higher bone mineral density in overweight people could be caused by the body-weight acting on the skeleton, or hormones, adipokines and signalling proteins produced by adipocytes, including leptin and adiponectin, which may act to contribute to bone mineral density through mechanisms not yet clarified [45,46].

The significant increase in haemoglobin during HR consumption was a positive observation, since HR components are plant foods, providing constituents, such as phytate, which can affect mineral bio-availability. The HR phytate: iron molar ratio was 8.28:1 mMol. Gibson et al. [47], described that the phytate: iron relationship should stay beyond 1:1. Instead, we observed increased haemoglobin concentration (Table 3), as was also found in a previous study in an animal model [48].

Erythrocyte zinc levels for both treatments were below the reference range [49]. Also, a negative association between BMI and erythrocyte zinc was observed. The physiological state of “sub-clinical inflammation” induced by overweight can increase levels of proinflammatory cytokines and cortisol generated by adipose tissue and overexpress metallothionein and zinc transporter Zip 14, increasing zinc concentration in the hepatocytes and decreasing the erythrocyte zinc concentration [50,51].

Some limitations of the study need to be clarified. Since it is a study of free-living individuals, adherence to a hypocaloric diet may not have been satisfactory to induce a negative energy balance. However, the recorded energy consumption presented less than 20kcal/kg/day (data not shown). The portion of HR daily consumed (20g) may have been insufficient to offer a high dietary fibre intake to modulate satiety and satiation as well as colonic effects to induce weight loss [10]. However, the choice was based on the usual intake in Brazil. Also, the intervention period may have been too short to induce significant metabolic changes.

The data in this study showed the potential of a mix of cereals, grains and seeds on weight and adiposity and allows analysis of the influence of such anthropometric variations in bone health. The importance of the study constitutes the scientific analysis of a product with great marketing around its effects on weight loss and improved health, without, however, showing data to prove this claim. The market for natural products and nutritional supplements has grown exponentially. It is necessary to study such nutrients or organic compounds on the metabolism to allow only those that have scientific evidence to be consumed by people, preventing health damage.

Conclusion

HR is an important vehicle of dietary fibre and micronutrients. Associated with a dietary energy restriction program, HR reduced the total fat and carbohydrate intake, increased the haemoglobin concentration and did not affect zinc metabolism or blood pressure. Despite changes in the pattern of abdominal fat distribution, there was no considerable weight loss.

Conflicts of Interest

The authors declare no conflicts of interest.

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