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Effects of Rapid Weight Loss following Iron Supplementation on Bone Mineral Density and Serum Osteocalcin Levels in University Wrestlers

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Abstract

This experiment is designed for bone health in wrestlers and martial arts athletes who lose weight with Rapid Weight Loss (RWL). To clarify the effect of iron intake on the relationship between weight loss and bone densityFor athletes using the RWL strategy, iron intake was observed for bone mineral density (BMD), serum osteocalcin (OC) level, and serum iron marker. This study was conducted on 23 wrestlers (13 males and 10 females). It was divided into the trial 1 (iron intake and weight loss) and the trial 2 (only weight loss). In addition, between the first and second experiments, a washout period was given for 3 weeks} in order to eliminate the medicinal effect. All measurements were performed in the same group. However, the OC levels were reduced following RWL. In case of the males, the intake group after weight loss demonstrated a significant increase in BMD ($p<0.05$). Correlation analysis of the data for the male group showed significant correlations between serum OC and iron contents ($p<0.05$), total BMD and TIBC(total iron binding capacity) ($p<0.05$), and transferrin and iron concentrations ($p<0.05$). In the female group, serum OC and iron ($p<0.01$), transferrin and TIBC(total iron binding capacity) ($p<0.01$) showed correlations. In addition, between serum OC and ferritin ($p<0.05$), total BMD and ferritin ($p<0.05$), TIBC and iron ($p<0.05$) were shown to have some degree of correlation in the global evaluations.

Key words: bone, osteocalcin, rapid weight loss, BMD, Wrestling

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Introduction

In many sports, body weight and body composition are very important factors (Sundgot-Borgen et al.,2013; Boileau et al.,2000). In almost all martial arts sports, athletes are classified according to body mass in order to compete more evenly, but athletes use rapid weight loss (RWL) strategies to compete against athletes of a weight class that is generally incompatible with their body type(Artioli et al., 2016; Franchini et al., 2012). Wrestlers take advantage of the shortcomings of the weight classification system to lose more than 10% of their body weight in a matter of days (Houston et al., 1981). Athletes use the following weight loss strategies to lose a lot of weight: a few days before starting weight loss, sweating, wearing a rubber suit, using a sauna, increasing the intensity and amount of training, dieting and fasting (Chiba et al., 1998; Katsumata et al., 2006; Kim et al., 2012; Shapses & Riedt, 2006).

RWL is commonly associated with the combat sports. The fast and aggressive weight loss associated with RWL can negatively affect athletes' performance(Chiba et al., 1998; Juanola-Falgarona et al., 2013; Khodaee et al., 2015), but since there are no rules restricting RWL, athletes and coaches are free to use them. This means that this method has the potential for abuse(Kim et al., 2012). And it should be used with caution as its application may cause long term health problems(Hunter et al., 2014).

Health problems associated with RWL include dehydration due to rapid weight loss, acute cardiovascular problems, acute and chronic hormonal imbalances, bone loss, suppressed immune function, increased susceptibility to infection, and increased risk of obesity after retirement(Artioli et al., 2016). RWL decreases both aerobic and anaerobic motor function. Aerobic dysfunction is dehydration, decreased plasma volume, increased heart rate, impaired hydrolysis, impaired thermoregulation, and muscle glycogen depletion. On the other hand, decreased anaerobic performance is mainly associated with decreased buffering capacity, glycogen depletion and hydrolysis disorders(Artioli et al., 2016).

It is widely accepted that sudden weight loss using the RWL method negatively affects bone density, but the negative effects associated with RWL remain somewhat ambiguous. Previous studies have reported bone deterioration on response to the rapid loss of large amounts of weight over very short periods of time(Choksi et al., 2018).

Athletes generally need the nutrients provided by a balanced diet to compete in optimal condition(Centi et al., 2013; Kim et al., 2018). Dietary restrictions or fasting during RWL can negatively affect the nutrition of athletes, which may have a negative impact on their overall health and performance. However, if the nutritional intake of an athlete is restricted, like what happens during RWL, there is the possibility of developing serious micronutrient deficiencies(Balogh et al., 2018). Among the micronutrients, iron is considered one of the most important for athletic nutrition and performance. Athletes whose iron status is sub-optimal may experience decreased athletic performance(Kim et al., 2012). In wrestling, iron is also an important factor when it comes to performance(Tayebi et al., 2017). Pathologically, iron deficiency causes several health problems, including anaemia, hyperlipidaemia, lipid peroxidation, and changes in the vitamin metabolism(Ghaleb et al., 2021). In a previous study, Katsumata et al. (2006) fed mice an iron-free diet to determine the association between bone density and dietary iron. Rats fed an iron-free diet were reported to experience decreased serum osteocalcin(OC) production, bone mineral density (BMD), and femur strength(Kanazawa, 2015).

Iron plays an important role in collagen synthesis in bones, where it acts as a cofactor in the hydroxylation of proline and lysine necessary for collagen stability and maturation(Xifra et al., 2018). Thus, iron deficiency appears to have a negative effect on bone production, but research data are insufficient and studies related to iron, BMD, and bone turnover indicators are still needed.

There are relatively few studies evaluating the link between iron deficiency and weight loss in humans. Moreover, far fewer studies have focused on iron and RWL. Therefore, it is clear that more research in this area needs to be completed.OC, which is a more specific marker of bone metabolism than serum alkaline phosphatase, is a non-collagen protein produced only in the osteoblasts making it a clear indicator of osteoblast activity in the clinical setting. The uniqueness of this marker means that serum samples may clearly reflect both OC function and bone turnover(Prouteau et al., 2006). Therefore, this study was designed to evaluate the relationship between RWL, BMD, and iron intake using serum OC, an index that specifically indicates bone metabolism, as the clinical readout. In addition, basic physical characteristics were also evaluated by dividing the region fat, tissue % fat, body fat mass, and tissue mass affected by weight loss into the whole body, arms, legs, and trunk.

Methods

Participants

This study population comprised of 23 elite wrestlers (13 males and 10 females) registered with the Korean Wrestling Association who had more than seven years of experience, All of them are wrestlers belonging to the university. All subjects received a comprehensive explanation of the research content, purpose, and associated procedures prior to participation and voluntarily consented to participate.

Ethical approval

This study was conducted according to the guidelines laid out in the Declaration of Helsinki, and all procedures involving human subjects were approved by the research ethics committee (approval no. 2-1040966-AB-N-01-20-1803-HMR-0992). Written consent was obtained from all subjects.

Experimental design

The study consisted of a single-blind test with two trials, including a 3-week washout period(Asemi et al., 2017), referred to as trial 1 and trial 2. Trial 1 consisted of an initial pre-test on day 1 where body composition, blood sampling, and a maximal incremental running test were all evaluated in an effort to determine the participant's baseline. This was then followed by a 7% weight loss over 7 days while consuming a natural . The participants were instructed to drink the (Spatone Water, Nelsons, UK) (Table. 1) containing 5 mg of iron and orange juice at 06:00 and 20:00 (two pouches per day) for the 1 week- weight-loss period. Trial 2 consisted of the same pre-test, followed by an approximately 7% weight loss over 7 days, only drinking orange juice. Both groups were then evaluated using a post-test regimen that evaluated the same markers determined in the pre-test. This evaluation was completed at 06:00 on day 7 for both trials. During the weight-loss period, subjects completed the training protocol two or three times per day, totalling approximately 5 h of exercise each day, and 30 h over the course of the week. During the washout period, participants were expected to control for both exercise and diet.

Weight reduction method

For this trial, it was important to develop a training program that reduced body weight, since we focused only on exercise intensity without dietary regulation. Trial 1 and Trial 2 used the same weight loss program. Diet supplements were completely banned, but additional food intake was not controlled. As such, the core strategies for the training program included weight loss while maintaining athletic performance. Thus we focused on exercises that would help to maintain or improve explosive strength, muscle strength, muscle endurance. And cardiovascular endurance. For the morning session, the main training program consisted

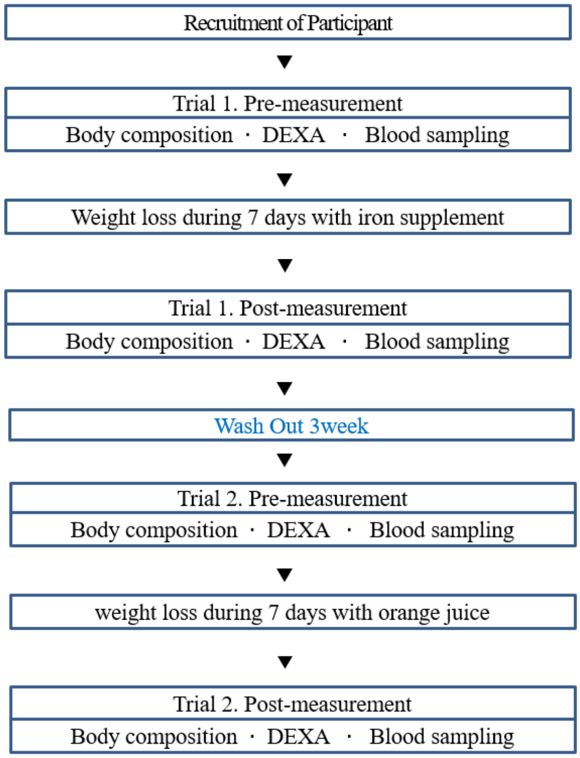

Figure 1. *Experimental desig*

of running and sprinting to increase cardiovascular endurance and muscle endurance. The sprint training, designed to improve explosive strength, was performed on a plain, a hill, and a step on Monday, Wednesday, and Thursday. Interval training and fartlek training were performed to improve cardiovascular endurance and muscle endurance on Tuesdays and Fridays. To reinforce this training, sub-training was completed after each training session. The afternoon sessions consisted of mat training and was designed to target physical fitness, skill development, sparring, and ground sparring to improve competitive strategy. In the evening sessions, the subjects performed weight training to maintain their strength on Tuesday and Thursday, but took a rest to recover on Monday, Wednesday, and Friday. The weight training program consisted of a power clean, dead lift, squat, and sit-ups to target the power zone in these athletes, including the lower back, abdominal region, and thighs.

Bone mineral density analysis

Bone mineral density (BMD) was evaluated using dual energy X-ray absorptiometry (DEXA). Our method relied on enCORE's Lunar Prodigy Advance version 16 program (Prodigy, Encore, California, USA). After measuring the whole body, the BMD was then determined for the arm, leg, and trunk. In addition, these readings were also used to determine the % of fat per region, the tissue fat content, total fat mass, and tissue mass, respectively. Wrestlers were measured according to expert instructions after changing clothes and removing all metal before the DEXA measurement.

Blood sampling and analysis

Blood samples were collected from the brachial vein four times (during each trial’s pre- and post-test: before body composition measurement) blood is collected with a 5ml Vaccume tube (vacuum blood collection tube, 1 serum tube) and A 22 gage needle was used to draw blood from the brachial vein. Plasma aliquots were centrifuged and then stored in a freezer for subsequent analyses. All eight analytes, including OC and the iron metabolism index, were analysed by Green Cross Laboratories in Korea.

Statistical analysis

Statistical analyses were performed using SPSS Statistics for Windows (version 25.0; IBM Corp., Armonk, NY). All data are presented as the mean ± SD. Two-way repeated-measures ANOVA with post-hoc evaluation by paired t-test was then used to identify the differences in blood parameters between the pre- and post-test samples in both the supplemented and non-supplemented trials. Data around the various iron factors, BMD, and OC were also evaluated for correlations and statistical significance was set at $P < 0.05$.

Results

Participant body weight analysis

Table 2 shows the basic characteristics of each participant and highlights the changes in these baseline values following natural action. This data includes their age, height, body weight and composition. The average body weight for the male group was 73.2 ± 7.1 kg and 73.7 ± 6.7 kg in the iron-supplementation and placebo group, respectively. After the rapid-weight-loss program, the body weight average decreased by 6.2% and 6.1% in the iron-supplementation and placebo group, respectively. In the female cohort, the average body weight was 65.2 ± 7.7 kg and 67.8 ± 6.9 kg in the iron-supplementation and placebo group, respectively, which decreased by 5.8% and 6.2% after implementing the rapid-weight-loss program. There were no significant body weight changes between the first and second trials over the 3-week washout period, indicating that our weight-loss program was effective in both trials.

Table 1. Nutritional Composition of iron supplement

Serving size	Amount per serving	% Daily value
Kcal	0kal	
Carbohydrate	0g	0%
Protein	0g	0%
Fat	0mg	0%
Sodium	0mg	0%
Iron	5mg	42%

Haematological factors

Table 2 shows the changes in the haematological factors in these subjects. The male group experienced no significant increase in the number of red blood cells (RBCs) in response to iron-supplementation but the number of RBCs was decreased versus the baseline in the placebo group following a week of RWL. Both the Hb and haematocrit (Hct) levels increased significantly in the iron-supplementation group ($p<0.05$, and $p<0.01$, respectively), but decreased in the placebo group. In the female participants both the Hct ($p<0.01$), as the RBC count (from 4.48 ± 0.22 to 4.58 ± 0.30) increased in response to action. Changes in the other blood cell types (WCV, platelets, WCV, MCH, and MCHC) were not significant in any of the group following RWL.

Analysis of the blood iron factors

Serum iron concentration increased in both male and female. Serum iron increased in the iron-supplementation group by 20.53% and 19.41% in males and females, respectively and 4.73% and 13.71%, compared to the Placebo group. The only significant difference was observed in males ($p<0.05$) where both transferrin ($p<0.001$) and TIBC ($p<0.001$) increased in the iron-supplementation group. The iron metabolism index (transferrin, ferritin, and TIBC) was not significantly different in female after RWL and Hp was

Table 2. Basic characteristics of participants and complete blood cell types

Factor	Male				Female			
	Iron		Placebo		Iron		Placebo	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Age (years)	21.6± 0.8				20±1			
Height (cm)	169.8±4.7				162.4±6			
Body weight(kg)	73.2±7.1	68.3±6.2	73.7±6.7	69.3±6.1	65.2±7.7	61.4±7.3	67.8±6.9	63.7±9.5
SMM (kg)	35.4±3.8	33.3±3.1	34.9±3.8	33.6±2.7	25.8±2.3	24.5±2.1	27.1±1.7	25.8±2.9
Fat mass (kg)	12.3±2.9	9.8±1.9	12.7±3.1	10.1±2.1	18.1±3.9	16.7±3.9	19.6±4.1	17.1±4.6
Body fat (%)	16.2±2.7	14.6±2.2	17.5±2.8	14.2±2.3	27.2±2.9	26.2±2.9	28.6±3.	26.7±3.5
BMI (kg/m^2)	25.7±2.1	24.1±1.2	25.5±2.1	24.4±1.2	24.7±2.3	23.6±1.9	25.9±2.2	24.3±2.7
WBC (106/lL)	6.64±0.12	7.28±1.05	7.18±1.67	6.56±1.34	7.49±0.86	8.36±1.41	7.89±2.02	6.74±0.43
RBC (106/lL)	5.11±0.12	5.26±0.13	5.32±0.12	5.06±0.23	4.48±0.22	4.58±0.30	4.91±0.35	4.82±0.36
Hb (g/dL)	15.61±0.39	16.03±0.39 *	16.28±0.66	15.30±0.72	14.36±0.43	13.88±0.62	14.78±1.43	13.96±1.12
Hct (%)	47.21±1.22	48.13±1.25 **	49.18±1.4	47.3±1.5	44.74±1.37	46.38±2.52 **	45.37±3.75	44.99±2.65
Platelet (103/lL)	300.69±34.23	316.76±42.88	309.53±46.39	294.92±59.72	339.1±32.99	306.8±52.71	295.6±50.09	2982.5±29.46
MCV (fL)	92.29±2.45	91.63±2.44	92.46±2.06	91.33±2.19	92.71±2.28	91.63±2.44	93.21±2.37	92.19±1.43
MCH (pg)	30.43±0.93	30.51±0.89	30.63±1.10	29.54±1.23	30.14±1.16	30.41±0.93	30.56±1.22	29.01±1.17
MCHC (%)	33.08±0.29	33.28±0.66	33.11±0.59	33.35±0.9	32.42±0.65	32.87±0.7	32.77±0.75	31.46±0.9

Values expressed as mean±SD*: *p*< .05, **: *p*< .01 vs Pre by t-test; SSM, Skeletal muscle mass; BMI, Body mass index; WBC, White blood cell; RBC, Red blood cell; Hb, Hemoglobin; Hct, hematocrit; MCV, Mean Corpuscular Volume; MCH, while Cell Volume; MCHC, Mean Corpuscular Hemoglobin Concentration; Fe, Iron; TIBC, Total Iron Binding Capacity; Hp, Haptoglobin

not significantly different in any of the group following RWL<Figure. 2>

DEXA and OC analysis

Table 3, shows the changes in BMD in these topics. In the placebo group, men's BMD changed from 3,183.8 ± 235.6 ~ 3076.6 ± 266.9 after weight loss, and female's BMD from 2,666.4 ± 252 ~ 2675.9 ± 226.8. In the iron group, male BMD changed from 3235.8 ± 313.3 ~ 3075.9 ± 320.7 in weight loss, and from 2592.5 ± 314.2 ~ 2594.9 ± 298.3 in the female group. Regardless of the subjects' intake, the male and female group did not experience a significant increase in the number of BMDs. The changes in serum OC in these subjects. In placebo group, the number of serum OC decreased compared. in both male and female. The male group had a significant decrease in serum OC levels in response to weight loss. Serum OC decreased from 21.1 ± 7.5 to 18.1 ± 3.6 (14.21%). In the female group, Serum OC decreased to 19.1 ± 4.5 ~ 14.8 ± 2.8 (22.51%). In males in the iron group, the level of serum

Table 3. *The results of DEXA analysis*

Factor	Male				Female			
	Iron		Placebo		Iron		Placebo	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Arms BMD	450.40 ± 39.95	454.86 ± 59.30	444.94 ± 46.24	450.28 ± 51.48	329.74 ± 27.70	324.25 ± 43.02	329.54 ± 40.47	324.87 ± 46.06
Legs BMD	1110.72 ± 113.89	1129.93 ± 138.48	1107.50 ± 121.68	1115.65 ± 118.04	886.49 ± 92.22	884.83 ± 94.50	905.40 ± 101.28	853.18 ± 78.42
Trunk BMD	971.28 ± 106.32	1001.20 ± 120.36	959.47 ± 105.15	972.50 ± 98.45	828.49 ± 111.91	826.19 ± 108.57	809.43 ± 111.72	824.03 ± 98.87
Total BMD	2995.95 ± 281.72	3146.43 ± 326.67	3106.99 ± 342.49	3050.01 ± 15.21	2580.76 ± 292.65	2562.30 ± 302.98	2663.06 ± 345.03	2539.13 ± 308.25
Arms tissue mass	9136.63 ± 821.72	9217.53 ± 1309.03	9033.06 ± 922.19	9045.89 ± 992.15	7399.14 ± 611.87	6975 ± 905.01	7318.27 ± 983.09	7322.60 ± 965311
Legs tissue mass	22781.82 ± 2323.98	22961.34 ± 2972.54	22343.99 ± 2460.51	22443.85 ± 2208.20	21069.22 ± 3342.91	20360.76 ± 3411.27	21352.68 ± 4159.66	20107.99 ± 3770.18
Trunk tissue mass	31667.62 ± 3708.87	31608.28 ± 3970.79	31146.31 ± 3293.01	31261.99 ± 2926.35	29322.62 ± 4162.01	28388.97 ± 3797.02	29220.98 ± 5362.65	29673.14 ± 4625.91
Total tissue mass	68023.57 ± 6832.71	68263.37 ± 8467.95	66947.83 ± 6739.40	67093.36 ± 6054.91	61792.05 ± 7926.36	59788.92 ± 8085.66	62021.70 ± 10606.26	61144.99 ± 9332.11
Arms tissue %fat	0.16 ± 0.02	0.17 ± 0.03	0.16 ± 0.02	0.16 ± 0.02	0.28 ± 0.03	0.29 ± 0.03	0.29 ± 0.03	0.29 ± 0.3
Legs tissue %fat	0.18 ± 0.02	0.19 ± 0.03	0.18 ± 0.02	0.19 ± 0.02	0.30 ± 0.03	0.30 ± 0.03	0.31 ± 0.03	0.30 ± 0.4
Trunk tissue %fat	0.17 ± 0.04	0.19 ± 0.04	0.17 ± 0.03	0.18 ± 0.03	0.29 ± 0.04	0.28 ± 0.05	0.29 ± 0.04	0.28 ± 0.4
Total tissue %fat	0.18 ± 0.02	0.19 ± 0.03	0.18 ± 0.02	0.18 ± 0.02	0.29 ± 0.03	0.28 ± 0.04	0.29 ± 0.03	0.28 ± 0.3
Arms fat mass	1504.66 ± 240.89	1605.89 ± 349.16	1427.68 ± 243.63	1465.75 ± 245.68	2051.41 ± 373.36	2047.95 ± 372.25	2158.18 ± 457.36	2144.97 ± 421.42
Legs fat mass	4187.15 ± 692.43	4442.56 ± 897.73	4104.68 ± 667.38	4195.86 ± 636.04	6309.98 ± 1539.37	6264.17 ± 1571.43	6622.21 ± 1831.03	6029.1 ± 1795.17
Trunk fat mass	5608.17 ± 1582.66	6034.58 ± 1760.83	5430.94 ± 1361.45	5520.84 ± 1125.25	8696.14 ± 2145.90	7977.31 ± 2310.45	8569.01 ± 2582.47	8329.20 ± 2385.14
Total fat Mass	12204.10 ± 2398.90	12997.30 ± 2949.40	11864.08 ± 2191.97	12068.45 ± 1840.96	17908.09 ± 3960.56	17167.63 ± 4111.25	18233.92 ± 4793.69	17360.61 ± 4480.60
Arms region % fat	0.16 ± 0.02	0.17 ± 0.03	0.15 ± 0.02	0.15 ± 0.02	0.28 ± 0.03	0.29 ± 0.03	0.29 ± 0.03	0.28 ± 0.4
Legs region % fat	0.17 ± 0.02	0.18 ± 0.03	0.17 ± 0.02	0.18 ± 0.02	0.28 ± 0.04	0.27 ± 0.05	0.28 ± 0.04	0.27 ± 0.4
Trunk region % fat	0.17 ± 0.03	0.18 ± 0.04	0.17 ± 0.03	0.17 ± 0.02	0.28 ± 0.03	0.27 ± 0.03	0.28 ± 0.03	0.27 ± 0.3
Total region % fat	0.17 ± 0.02	0.18 ± 0.03	0.17 ± 0.02	0.17 ± 0.02	0.28 ± 0.03	0.27 ± 0.03	0.28 ± 0.03	0.27 ± 0.3
Osteoclacin	22.8 ± 6.3	24.2 ± 8.3*	21.1 ± 7.5	18.1 ± 3.6*	19.4 ± 4.7	16.3 ± 3.1*	19.1 ± 4.5	14.8 ± 2.8*

Values expressed as mean±SD*: $p < .05$ vs Pre by t-test; BMD, Bone mineral density
BMD(g/cm^3) / tissue mass(g) / %fat(%) / fat mass(g) / region % fat(%)/ Osteocalcin(ng/ ml)

OC increased to 22.8 ± 6.3 ~ 24.2 ± 8.3 (6.14%), In the case of female, serum OC decreased to 19.4 ± 4.7

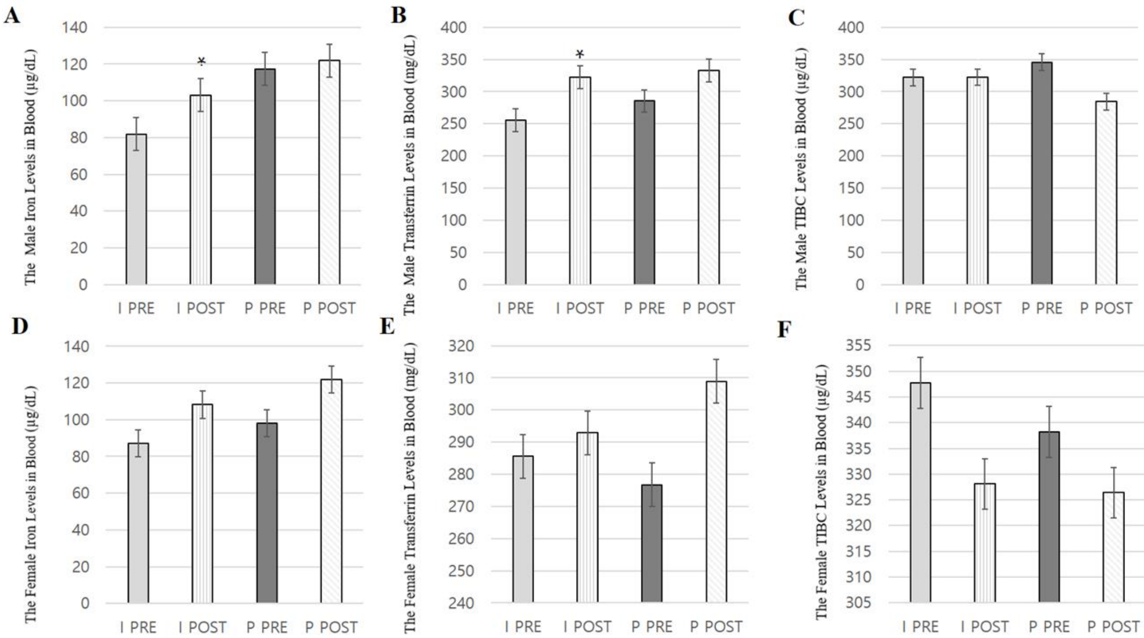

***Figure 2**. Analysis of the blood Iron factors (A) The Male Iron Levels in Blood, (B) The Male Transferrin Levels in Blood, (C) The Male TIBC Levels in Blood, (D) The Female Iron Levels in Blood, (E)The Female TIBC Levels in Blood, (F); I PRE, Iron pre-test; I POST, Iron post-test; P PRE, Placebo pre-tes; P POST, Placebo post-test*

~ 16.3 ± 3.1 (14.65%), but the reduction was less than that of the placebo female group (22.51%). Significant differences between the placebo group and the iron group after weight loss were found in both male($p<0.05$)($p<.048$) and female($p<0.05$)($p<.021$).

Correlation analysis

Each parameter used in the correlation evaluations are shows in Table 4. In the male group, serum OC and iron ($p<0.05$), transferrin and iron, and total BMD and TIBC ($p<0.05$) were all shown to demonstrate high correlation values. The R-value between the serum OC and iron concentrations was 0.278, suggesting that iron intake affects serum OC. The R-value for transferrin and iron was 0.341, and the R-value for TIBC and total BMD was 0.286. No significant differences were observed for any of the other parameters evaluated in this study. In the female group, the strongest associations were between serum OC, iron ($p<0.01$), and transferrin and TIBC ($p<0.01$). These results were similar to those of the male group and we were able to identify positive correlation coefficients for each of these parameters. Serum OC and iron showed a strong correlation in these samples with an R-value of 0.420. And the transferrin and TIBC values were equally convincing with an R value of 0.474.

When evaluated as a global data set several other factors were also shown to share some correlation including serum OC and transferrin ($p<0.05$), total BMD and ferritin ($p<0.05$), iron and TIBC ($p<0.05$). The R-value for the serum OC and transferrin was -0.368. And while the R-value for total BMD and ferritin was -0.336. The R-value for iron and TIBC was also negative at -0.323, suggesting that in all three cases there was a negative correlation between these values. No significant differences were observed among the other parameters.

Discussion

This study was designed to determine the effect of

Table 4. Correlation between OC, BMD and Iron factor

Male \ Female	Serum OC	Total BMD	Iron	Transferrin	Ferritin	TIBC
Serum OC		.053 (.747)	.420** (.007)	-.368* (.021)	-.173 (.285)	.091 (.582)
Total BMD	-.188 (.181)		-.026 (.873)	-.148 (.361)	-.336* (.034)	-.234 (.146)
Iron	.278* (.046)	-.111 (.433)		-.244 (.129)	.098 (.546)	-.323* (.042)
Transferrin	-.166 (.413)	-.032 (.819)	.341* (.013)		-.017 (.919)	.474** (.002)
Ferritin	-.122 (.387)	.069 (.625)	-.080 (.574)	-.070 (.623)		-.198 (.104)
TIBC	.163 (.247)	.286* (.040)	.-.070 (.621)	.013 (.926)	-.225 (.108)	

Values expressed as mean±SD *: p< .05, **: p< .01; OC, Osteoclacin; BMD, Bone mineral density, TIBC, Total Iron Binding Capacity

iron intake on serum OC levels and BMD in wrestlers undertaking RWL. This RWL was shown to be associated with changes in BMD, haematological characteristics, iron metabolism, serum OC. And each of these changes were shown to respond to action. During one week of weight loss, there were no significant changes in the athletes' BMD. And there were no changes in the BMD in the trunk, or the arms and legs. Previous data suggests that there may be a link between weight loss and diet influenced BMD(Ihle & Loucks, 2004; Wei & Karsenty, 2015), with significant weight loss over a short period of time likely to exert some negative impact on BMD in wrestlers; however, our evaluations revealed no significant changes in BMD in either male or female regardless of iron intake. In addition, there were no significant differences in the global fat deposition, tissue fat%, fat mass or tissue mass nor any of these parameters in the arm, leg, or trunk. Athletes can maintain and increase BMD by increasing resistance training or by performing additional weighted exercises. Therefore, it is possible that this impacted the athletes' BMD in this experiment. It is believed that this is the reason that the athletes perform weight training exercises like the power clean, deadlift, sprints level ground, stairs, and hills. In addition, because of the short-term weight loss peculiar to RWL, unlike other forms of weight loss (Ikeda et al., 2002; Shapses & Riedt, 2006), the observation period was very short, so it is possible that there was not enough time to observe a change in bone density. During RWL, the athletes' iron intake had no effect on BMD. Previous weight loss-related studies have used much longer observation periods, at least 3 weeks to 6 months, likely to allow for the evaluation of more long-term changes in the BMD parameters (Centi et al., 2013; Chiba et al., 1998). However, our study used a much smaller observation period in order to evaluate only the direct impact of RWL. The fact that the type of exercise and the length of the observation may counteract BMD deficiencies or be insufficient for obvious BMD initiation we cannot definitively conclude whether RWL may have a lasting negative impact on BMD(Centi et al., 2013; Chiba et al., 1998; Franchini et al., 2012).

Although iron did not have any effect on BMD, it was observed that OC levels significantly increased following iron intake. Serum OC decreased after weight loss in both the male and female placebo group, suggesting that short-term weight loss such as RWL decreases OC levels. Conversely, in the iron group, the OC content in the male group significantly increased. In the case of the female subjects there were differences in the extent of the OC reduction depending on the presence or absence of iron. The placebo group who did not consume iron had a 22.5% decrease in serum OC after weight loss however serum OC levels were still reduced by 16% in female group who took iron. In other words, iron intake during weight loss seems to mitigate or prevent the decrease in serum OC levels in males and to a lesser extent prevents these reductions in females. This is thought to be linked to the fact that OC and iron are closely associated in the energy metabolism, although these relationships have not been identified in bone. In our results, the decrease in OC in the placebo was the opposite of the trend observed during normal weight loss. OC levels increased after 4 months of diet in obese male subjects without diabetes(Albadah et al., 2015). Other studies have produced a similar trend to ours where OC decreases when evaluated in subjects using a combination of diet and exercise to lose weight(Ihle & Loucks, 2004; Yamanishi et al., 2003). Taken together, the above results suggest that serum OC generally increases with healthy weight loss. However, if you lose weight in a short period of time and restrict your diet you will experience a decrease in OC Secretion. This phenomenon is thought to be due to the newly described endocrine function of bone tissues. OC is involved in energy metabolism and has a well-defined function as a metabolic hormone. OC functions to decrease glucose concentration. And increase pancreatic beta cell proliferation, insulin secretion, and sensitivity. Another study has shown that OC acts directly on insulin levels. Also increases adiponectin in fat cells involved in glucose metabolism(Zoch et al., 2016). Trial 2, the decrease in serum OC seems to be due to reduced food intake or fasting designed to augment the RWL program. However, since there were no dietary restrictions or fasting controls in this study it is necessary to expand these evaluations to include a new study which strictly controls the dietary portion of these programs(Albadah et al., 2015; Buday et al., 2013; Centi et al., 2013; Jensen et al., 2001; Papageorgiou et al., 2018; Villareal et al., 2006).

OC is only produced in the osteoblasts. it is presumed to act as a hormone in a manner similar to those described for leptin and adiponectin. OC is synthesised only in the bone, but exists in the serum. its mechanism is currently unknown, also its role appears to be regulated via carboxylation. For example, carboxylated OC increases the expression of β-cells, which increases adiponectin expression in adipose tissue. The biological activation of OC is achieved by removing the carboxylated residues of OC(Zoch et al., 2016); in other words, depending on the circumstances, OC may be involved in energy metabolism. in addition, This may change depending on weight loss or diet. Iron is also involved in the energy metabolism(Ikeda et al., 2002). However, there is currently no direct evidence linking iron and OC under these circumstances. Iron negatively affects both bone overload and iron deficiency and requires optimisation to support bone homeostasis. In a previous study, the levels of serum OC in rats fed iron-deficient diets decreased(Ikeda et al., 2002). Conversely, the levels of OC in human osteoblasts overloaded with iron decreased(Bagchi et al., 2018). Overall, these studies confirm that OC is affected by iron levels but the exact mechanism remains unknown(Balogh et al., 2018; Franchini et al., 2012; Ikeda et al., 2002).

Improvement at the psychological level was also found with the exercising group experiencing lower levels of depression, fatigue, anger and tension and higher levels of vigour after the 19 week exercising period. On the other hand after the same period of time, the control group had not improved their mood states outcome and had even increased their confusion and decreased their vigour levels. The influence of exercise on mental well-being has now been the subject of several studies (reviewed by Fox, 1999) that suggest that aerobic and resistance exercise enhances mood states and may even improve cognitive function in older adults. These results are in accordance to those found in the present study and seem to indicate that exercise may have important benefits at the psychological level in elderly subjects, not only by enhancing the mood states but also by preventing their deterioration with time.

There was a significant correlation between serum OC and iron levels in both male and female participants which is similar to other published data(Choksi et al., 2018; Ghaleb et al., 2021). It is thought that there is a close relationship between serum OC and iron. In addition,, significant differences were observed in total BMC and TIBC in male. This phenomenon can be explained by studies showing that serum iron levels affect bone health(Pettersson et al., 2013), and studies have shown that serum TIBC levels change annual BMD in female(Jensen et al., 2001; Kim et al., 2020). In addition, we observed some correlation between transferrin and iron concentrations with these correlations reaching significance in the female participants. The female group also reported some correlation between total BMD and ferritin, iron and TIBC, and transferrin and TIBC. In the case of serum OC and transferrin, these association can be explained by a previous study(Katsumata et al., 2006) that showed that serum transferrin receptor levels are closely associated with total and carboxylated OC concentrations in the serum. In addition, the association between total BMD and ferritin levels can be explained by a previous study (Babaei et al., 2018) evaluating BMD and serum ferritin in the elderly. The remaining iron, transferrin, and ferritin correlations are likely the result of the known relationships between these parameters, which have been described in previous studies.

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Conclusion

In conclusion, short-term weight loss does not appear to affect BMD, but micronutrient intake does exert some negative effects on the serum OC levels. In addition, we found that action alleviated these decreases in serum OC concentration. This phenomenon suggests that athletes must consider their nutrient and micronutrient requirements when undertaking a weight loss regimen. This study also revealed that RWL induced more noticeable effects in serum OC concentration than BMD. This is notable, as OC affects not only the bones, but also other physiological functions, and it is believed to act as an important metabolic hormone, which suggests that its secretion may have a notable impact on the uptake of the aforementioned micronutrients. In addition, although the BMD levels and the global OC levels did not decrease in these athletes, the localised reductions in OC levels may have a significant long term impact on BMD following habitual RWL. Therefore, we cannot conclusively determine that short-term weight loss does not negatively affect the bones. As with previous studies, coaches and athletes are aware of the risks of RWL, and athletes should consume adequate micronutrients to minimise damage to their body damage during periods of RWL. However, because iron overload is also harmful to health, an appropriate intake of iron should be set based on the extent of the weight loss goals.

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