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The impact of weight loss diet programs on anemia, nutrient deficiencies, and organ dysfunction markers among university female students: A cross-sectional study

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ABSTRACT

Practicing restricted weight loss diet programs (WLDPs) without proper supervision can result in nutritional deficiency, which can lead to the development of several nutritional disorders. The current cross-sectional study aimed to investigate the impact of WLDPs practiced by university female students on the prevalence of micronutrient deficiencies, anemia, and organs dysfunction, and to assess the association of identified anomalies with dieting practices and dietary habits of university female students. A total of 185 (105 dieting and 80 non-dieting) volunteers' female students at Al-Hussein Bin Talal University participated in this study. After the participants answered a questionnaire, blood samples were collected for hematological and biochemical analysis, and the body mass index (BMI) was determined. The results show that there were no significant differences between dieting and non-dieting groups in biochemical markers of kidney and liver functions as well as serum levels of copper, zinc, and folate. On the other hand, dieting participants exhibited significantly lower level of hemoglobin, serum ferritin, iron, and vitamin B12 than encounter group ($p<0.05$). Attempting WLDPs significantly increased the prevalence of anemia (46.7%), iron deficiency (57.1%), and iron deficiency anemia (IDA) (41.9%), comparing to non-dieting students (28.7%, 33.8%, and 15.0%, respectively) ($p<0.005$). Chi-square test showed that the development of anemia among dieting girls was significantly dependence of several factors including BMI category, source and duration of the diet programs, and skipping breakfast ($p<0.05$). In conclusion, young girls attempting WLDPs without professional guidance are more prone to the risk of nutrients deficiencies and the development nutritional disorders like IDA. An educational program should be employed to teach young females on when and how to adopt healthy WLDPs.

Keywords: weight-loss diet programs, anemia, IDA, dietary habits, BMI, skipping breakfast

INTRODUCTION

Obesity is a growing worldwide health problem, which is engaged in the development of various chronic diseases such as diabetes mellitus, dyslipidemia, and cardiovascular diseases [1, 2]. Many diet programs have been scientifically evidenced to reduce weight and decrease risk of obesity related health problems along with safeguarding individuals from nutrient deficiency [3]. On the other hand, following restricted and uncounted diet strategies can result in nutrient deficiency, which is responsible for a group of health disorders including anemia, osteoporosis, and neurological diseases [4-7].

According to the World Health Organization (WHO), the healthy body mass index (BMI) is 18.5-24.9 kg/m^2, while an individual with BMI 25.0-29.9 kg/m^2 is considered overweight, and obese person is with BMI≥30.0 kg/m^2 [8]. For overweight and obese individuals, it is highly recommended to decrease their body weight through adopting lifestyle modification including lowering calories intake concomitant with increase physical activities [9]. However, many young people, especially females, with normal BMI misperceive their body weight due to dissatisfaction with body image in addition to psychological and socio-cultural factors [6, 10, 11]. Those people usually desire to be thinner by practicing unhealthy diet regimens that cause inappropriate weight reduction and nutritional disorders [12-14]. In fact, several reports have revealed the link between practicing unhealthy weight loss diet programs (WLDPs) and the development of physiological and psychological disorders among population of developed countries [6, 7, 13, 15]. However, studies investigating the harmful effects of attempting WLDPs on the health of subjects in developing countries remain largely limited [11].

Unhealthy WLDP includes extreme calorie restriction and nutritional imbalance, skipping meals, taking laxatives, vomiting inducers, or diuretics, taking non-prescription diet pills/supplements, and practicing vigorous exercises [16, 17]. The severe negative energy balance caused by the very-low-calorie diets is responsible for a variety of health complications including cholelithiasis, loss of lean body mass, ketosis, and increased serum uric acid concentrations [18]. Likewise, it was found that following long term high-fat-low-carbohydrate diet causes anemia, metabolic acidosis, and reduces antioxidant enzyme level in rats [19]. A study revealed the deficiency of 27 micronutrients among individuals attending four popular diet programs practiced in the USA [20]. A significant alteration in micronutrient profile was found among subjects after experience of two months period of commercial WLDPs in multi-centre randomized controlled trial [21]. Furthermore, the extreme weight loss strategies used by skilled jockeys in Korea result in insufficient nutritional intake and a considerable decrease in bone mineral density, which is implicated on the aggravation of injuries during horse races [22].

Adults suffering from nutritional disorders may exhibit severe organ dysfunction as well as atypical signs and symptoms of common health problems, which may be misdiagnosed or sub-optimally treated [23]. For example, insufficient intake of iron, vitamin B12, folate, as well as caloric and protein restriction, can cause nutritional anemia, which is manifested by low Hb concentration (<13.0 g/dL for male, <12.0 g/dL for female) [24]. In fact, the adult females are more susceptible to anemia than males due to the blood loss during menstrual cycle [25]. According to the recent review of the nutrition situation among Jordan population, the prevalence of anemia among women of reproductive age showed escalating trend, raising from 26% in 2002 to 43% in 2017 [26]. Moreover, a previous national micronutrient survey demonstrated that 30.6% of non-pregnant women of reproductive age suffer from anemia, 35.1% had iron deficiency, and 19.8% with iron deficiency anemia (IDA) [27].

According to our recent survey, a sizable proportion (46.2%) of Al-Hussein Bin Talal University (AHU) students practiced different WLDPs, particularly female students who represented 91.5% of dieters [28]. The study revealed data on the motivating purposes behind the students' choice to attempt WLDPs, the variances in the sources from which the students acquired information about their selected programs, and most crucially, their eating habits while following the weight-loss diet programs, and the common symptoms reported by students engaging in these diet programs. Furthermore, the study demonstrated a strong association between skipping breakfast meal and the prevalence of irregular and painful periods among dieting female students. Consequently, the purpose of this study was to investigate the impact of various WLDPs attempted by female students on hematological and biochemical parameters, as well as to examine the relationships between BMI values, eating habits, and reported symptoms with aberrant biochemical and hematological findings.

MATERIALS AND METHODS

Participants

A total of 185 volunteers' female university students, from AHU, participated in this cross-sectional study. The participants were 105 female students attempting WLDPs and 80 age matched female students as a control group who were not following any WLDPs. The criteria for including the participants were to be female within 18-25 years age, free of any diagnosed health problem, not taking any medication or nutrient supplements except those were taken as purposely for reducing weight as part of the adopted program, non-pregnant or lactating woman, and for testing group they should have attended the WLDPs for at least three months because a short period of attempting diet program might not be enough to be investigated for its impact on health and nutritional status.

Questionnaire and BMI

The participants were asked to answer the questionnaire, which includes parts of sociodemographic data (age, marital status, and university college) and a direct question to indicate if the participants were attempting WLDPs or not. For those who attempting WLDPs, they were asked to continue answering questions regarding their eating habits and the components of the practiced WLDPs. In this section, participants were asked to identify their primary goal for engaging in WLDPs, whether it is to improve body image, prevent disease, or facilitate mobility. The second question was inquiry about the source of the attempted WLDPs followed by participants, whether it was from a dietitian, a website, friends and relatives, a self-planned program, or any other source. In addition, the level of exercise incorporation in the followed dieting programs was questioned by asking dieting participants to score their daily exercise out of five in which the minimum score one was given for the choice never-rare, score two for seldom-occasionally, score three for sometimes, score four for often-frequently, and score five for usually-always. The duration since attempting the program was determined starting from three-four months, until the period >six months. Further question was incorporated to ask about the amount of daily intake of pure water by dieter students, given five answer categories including <1 L, 1-2 L, 2-3 L, 3-4 L, and >4L. Dieting students' commitment to counting their daily calorie intake was evaluated by assigning a score of one to the choice never-rare, score two to seldom-occasionally, score three to sometimes, score four to often-frequently, and score five to usually-always. Involvement of taking drugs or herbal products as part of the WLDP was questioned in an enquiry to choose between using herbal products, non-prescribed drugs, prescribed drugs, or nothing at all. Furthermore, a diet program requiring skipping one or more of the major meals was determined by using a multiple-selections question with answer options including skipping breakfast, skipping lunch, skipping dinner, and taking all meals. Lastly, another multiple-selections question was used to ask the participants if any of the selected symptoms, including fatigue, loss of concentration, irritable mode, falling hair, painful extremities, irregular and painful period, recurrence of infections, or nothing were observed while practicing their WLDPs.

Each questionnaire was given a code number, which was matched with the code recorded on the blood samples. The questionnaire was built in Arabic language as it is as the public spoken language. It was obtained from our previous survey investigated the extent to which AHU students were adopting WLDPs and the variances in the information sources, strategies, eating habit in addition to diet associated symptoms reported by the participants [28]. As described in our above-mentioned previous work, three experts in public health research from three different universities confirmed the questionnaire's validity and reliability. The committee was asked to review the questions for inclusion in the final questionnaire for relevance. All their suggestions and corrections were incorporated into the questionnaire's final draft. The weight and height were measured by the research assistants and recorded on the questionnaire of each subject. BMI values were detected by dividing weight (kg) by height

Table 1. Distribution of the participant's BMI categories, colleges, and marital status among dieting and non-dieting groups

Variables		Dieting n/105 (%)	Non-dieting n/80 (%)	Total n/185 (%)	p-value
College	Health	50 (47.6%)	37 (46.3%)	87 (47.0%)	0.853[c]
	Non-health	55 (52.4%)	43 (53.8%)	98 (53.0%)	
Marital status	Single	100 (95.2%)	78 (97.5%)	178 (96.2%)	0.424[c]
	Married	5 (4.8%)	2 (2.5%)	7 (3.8%)	
BMI	Healthy	41 (39.0%)	67 (83.8%)	108 (58.4%)	<0.001[c]
	Overweight	41 (39.0%)	10 (12.5%)	51 (27.6%)	
	Obese	23 (21.9%)	3 (3.8%)	26 (14.1%)	
	Mean±SEM	26.37±0.45	23.02±0.33	-	0.000[t]
Age (years)	Mean±SEM	20.70±0.12	20.6±0.16	-	0.564[t]

Note. Data of BMI categories, colleges, and marital status were expressed as number (n) (% of n among dieting or non-dieting groups); data of BMI and age of groups members were expressed as mean±SEM; [c]Chi-square test was used to assess variables independence; [t]Independent t-test was used to compare the mean; & statistically significant difference or association were considered at p<0.05

squared (m^2) [29]. According to BMI values, participants were categorized as having a healthy BMI (18.5-24.9 kg/m^2), overweight (BMI 25.0-29.9 kg/m^2), and obese (BMI≥30.0 kg/m^2) [8].

Blood Sample Collection

For blood sample collection, the volunteers were asked to come to the medical center in AHU during the morning time (8.00-10.00 am) while they were fasting. Blood samples were collected into three distinct tubes through using standard procedure by qualified research assistants. EDTA tubes were used for hematological analysis, trace element tubes (gray capped) for copper (Cu) and zinc (Zn) analysis, and plain tubes (red capped) for biochemical analysis. Serum samples were obtained from trace element and plain tubes after centrifugation for five minutes at 3,000 RPM, and they were aliquoted in Ependorf® tubes and kept at -20 °C for analysis.

Hematological and Biochemical Analysis

Anemia profile tests including hemoglobin (Hb) concentration, packet cell volume (PCV), mean cell volume (MCV), mean cells hemoglobin (MCH), mean cells hemoglobin concentration (MCHC), red blood cells (RBC) count, red blood cells distribution width (RDW), and platelets (PLTs) count were performed within one-two hours of sample collection by using Sysmex automated hematology analyzer (KX-21N, Tokyo, Japan). On the other hand, levels of folate, vitamin B12 (VB12), and ferritin were measured using automated chemiluminescence immunoassay analyzer (CL-900i, Mindray, China). Female students with Hb levels <12.0 g/dL or ferritin levels <15.0 ng/mL were defined as anemic or iron deficient, respectively, while those with both abnormalities were designated as IDA [30, 31]. Additionally, kidney functions tests (KFT) including creatinine (Cr), blood urea nitrogen (BUN) and uric acid (UA); liver function tests (LFT) including alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (TB), direct bilirubin (DB), total protein (TP), and albumin (Alb) concentration; in addition to serum iron (SI) levels were measured in serum samples by using automated chemistry analyzer (BS-240 Mindray, China). Furthermore, serum levels of Zn and Cu were detected by flame atomic absorption spectroscopy (Agilent Technologies, the USA) according to the method described previously [32]. All analysis was carried out in the laboratories of the Medical Analysis Department in AHU.

Statistical Analysis

The data was presented as means ± standard error mean (SEM). The statistical package for the social sciences (SPSS) program version 25 was applied for statistical analysis. To compare the mean values of variables among two different groups, an independent t-test was used. Meanwhile, one-way analysis of variance (ANOVA) was used to compare the mean values of variables among multiple groups. Additionally, Chi-square test was used to assess the association of the occurrence of anemia with several variables. A p-value of <0.05 was considered for detecting the statistically significant differences and associations.

RESULTS

Demographic Data and BMI

A total of 185 female students, 18-24 years age, participated in the present study. Among them, 105 (age 20.71±1.2 years) were attempting WLDPs and 80 (age 20.60±1.4 years) were not adopting any diet program. As shown in **Table 1**, 87 (50 dieting, and 37 non-dieting) students were from health science colleges, while 98 students (55 dieting and 43 non-dieting) belonged to non-health colleges. Among the participants, there were only seven married students (five dieting and two non-dieting), and 178 (100 dieting and eight non-dieting) were singles. Majority of the participants (n=108, 58.4%) were having healthy BMI representing 39.0% of dieting group and 83.8% of control group. Meanwhile, the overweight and obese students accounted for 39.0% and 21.9% of dieting group and only 12.5% and 3.8% of non-dieting group, respectively, representing a total of 41.7% (27.6% overweight and 14.1% obese) of the whole participants. The statistical analysis revealed that there is a significant relationship between the students BMI and the decision of adopting WLDP (p<0.001). Likewise, the mean value of the calculated BMI was found to be significantly higher among dieting group (26.37±0.45) comparing to non-dieting group (23.02±0.33) with p<0.001. However, attempting WLDP was found to be independent of the field of college or marital status of the participants. Also, no significant difference was detected between the mean age of dieting (20.7±0.12) and non-dieting groups (20.60±0.16).

Biochemical Profile

To investigate the effect of adopted WLDPs on serum biochemical profile of the participants, the serum levels of KFTs (Cr, BUN, UA); LFTs (TB, DB, AST, ALT, TP, and Alb); and the trace elements (Cu and Zn) were analyzed, and the results were compared between dieting and non-dieting groups. As shown in **Table 2**, there were no statistical differences in the results of biochemical tests between the dieting and non-dieting groups.

Table 2. Biochemical profile of dieting and non-dieting female students

Tests		Dieting participants (n=105) [mean±SEM]	Non-dieting participants (n =80) [mean±SEM]	p-value
KFT	Cr (mg/dL)	0.76±0.01	0.74±0.02	0.541
	BUN (mg/dL)	12.00±0.30	11.50±0.30	0.318
	UA (mg/dL)	4.60±0.10	4.40±0.10	0.276
LFT	TB (mg/dL)	0.32±0.02	0.36±0.02	0.183
	DB (mg/dL)	0.16±0.01	0.17±0.01	0.419
	AST (U/L)	17.10±1.30	15.30±0.50	0.267
	ALT (U/L)	7.00±0.60	6.40±0.50	0.413
	TP (g/L)	80.10±0.80	80.80±1.40	0.702
	Alb (g/L)	48.10±0.50	49.00±0.80	0.332
Trace elements	Cu (µg/dL)	94.00±1.10	96.70±1.40	0.116
	Zn (µg/dL)	89.30±2.40	90.20±3.20	0.822

Note. Data expressed as mean±SEM; independent t-test was used to compare means; & statistically significant difference was considered at p<0.05

Table 3. Anemia profile of dieting and non-dieting female students

Test	Dieting participants	Non-dieting participants	p-value
Hb (g/dL)	11.9±0.20	12.4 ±0.20	0.015
RBCs count ($\times 10^{12}$/L)	4.5 ±0.04	4.6±0.04	0.335
PCV (%)	38.7±0.50	40.4±0.50	0.018
MCV (fL)	85.7±0.80	88.4±0.80	0.016
MCH (pg)	26.3±0.30	27.3±0.30	0.023
MCHC (g/dL)	30.6±0.20	30.8±0.20	0.405
RDW (%)	16.7±0.30	15.8±0.30	0.014
PLTs count ($\times 10^{9}$/L)	331.2±8.50	299.6±9.00	0.013
Ferritin (ng/mL)	17.5±1.60	23.9±2.10	0.013
Serum Iron (µmol/L)	12.3±0.70	15.5±0.80	0.003
VB12 (pg/mL)	347.6±12.00	395.5±14.80	0.012
Folate (ng/mL)	8.3±0.40	8.3±0.50	0.952

Note. Data expressed as mean±SEM; iIndependent t-test was used to compare means of diet group against non-diet group; & statistically significant difference was considered at p<0.05

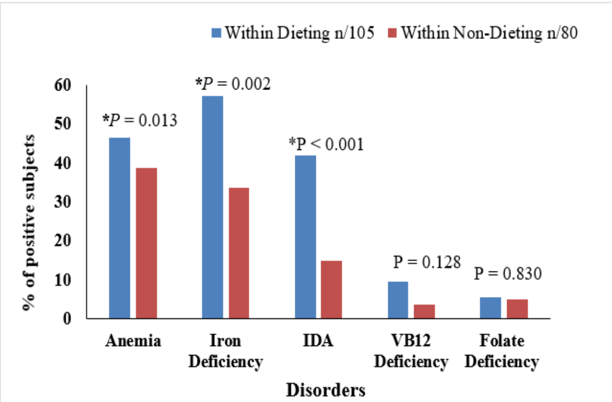

Figure 1. Prevalence of anemia, iron deficiency, IDA, V-B12 deficiency, and folate deficiency among dieting and non-dieting female students (data was expressed as percentage of positive cases of various nutritional disorders among dieting and non-dieting groups; Chi-square test was used to assess variables independence; & significant association was considered at *p<0.05). (Source: Authors' own elaboration)

Anemia Profile

The prevalence of anemia among the female students engaged in WLDPs was investigated and compared with non-dieting group. For this purpose, the results of Hb concentration, RBCs count, PCV, MCV, MCH, MCHC, RDW, and PLTs count in addition to serum levels of ferritin, SI, folate and VB12 were compared between dieting and non-dieting groups.

As presented in **Table 3**, dieting participants showed significantly (p<0.05) lower mean values of Hb concentration (11.9±0.2), PCV (38.7±0.5), MCV (85.7±0.8), MCH (26.3±0.3), ferritin (17.5±1.6), SI (12.3±0.7), and VB12 (347.6±12.0), compared to non-WLDPs participants (12.4±0.2, 40.4±0.5, 88.4±0.8, 27.3±0.3, 23.9±2.1, 15.5±0.8 and 395.5±14.8, respectively).

On the other hand, the mean values of RDW (16.7±0.3%), and PLTs count (331.2±8.5×10^9/L) were significantly higher (p<0.05) in dieting female group compared with control group (15.8±0.3%, and 299.6±9.0×10^9/L, respectively). Meanwhile, there were no significant differences between RBCs count (4.51±0.04×10^{12}/L vs 4.56±0.04×10^{12}/L), MCHC values (30.6±0.2 vs 30.8±0.2), and folate level (8.3±0.4 vs 8.3±0.5) between the two groups (p>0.05).

In addition, cross tabulation analysis of the distribution of anemia (Hb <12 g/dL), iron deficiency (Ferritin <15 ng/mL), IDA (Hb and ferritin <12 g/dL and 15 ng/mL, respectively), VB12 deficiency (VB12 <250 ng/mL), and folate deficiency (Folate <2 ng/mL) subjects revealed various degrees of the prevalence of these disorders within dieting and non-dieting groups (**Table A1** in **Appendix A**). The prevalences of anemia, iron deficiency, and IDA were found to be much higher among participants attempting WLDPs (46.7%, 57.1%, and 41.9%, respectively) compared with non-dieting control group (28.7%, 33.8%, and 15.0%, respectively) (**Figure 1**). Furthermore, Chi-square test analysis of the proportions of positive and negative cases showed significant association between participants attempting WLDPs and the development of anemia, iron deficiency, and IDA (p<0.005). However, there was no significant relationship between practicing WLDPs and the occurrence of VB12 or folate deficiencies (p>0.05).

To explore the key nutritional factors contributing to the development of anemia among dieting female students, the distribution of iron, VB12, and folate deficits among anemic and non-anemic dieters was demonstrated (**Table A2** in **Appendix A**). As shown in **Figure 2**, the vast majority of anemic

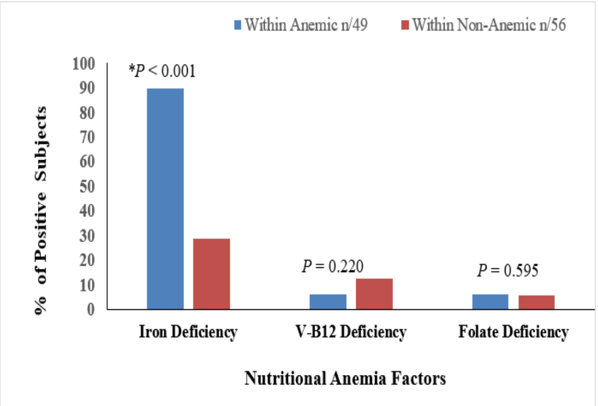

Figure 2. Percentages of iron, V-B12, and folate deficiencies among anemic and non-anemic dieting female students (data was expressed as percentage of positive cases of various nutritional deficiencies among anemic dieters and non-anemic dieters; Chi-square test was used to assess variables independence; & statistically significant association was considered at *p<0.05). (Source: Authors' own elaboration)

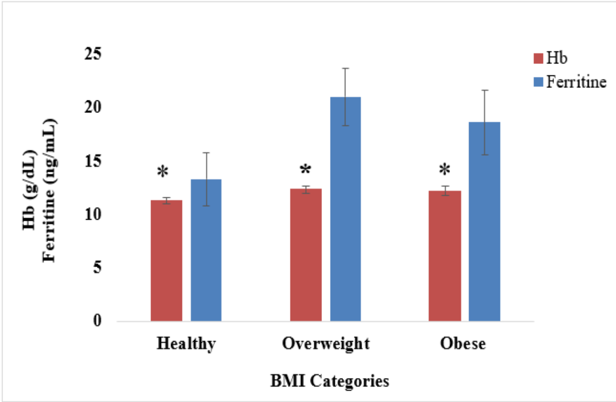

Figure 3. Hb and ferritin concentrations among BMI categories (healthy, overweight, and obese) of dieting students (data was expressed as mean±SEM; one-way ANOVA test was used to compare the means; & statistically significant difference was considered at*p<0.05). (Source: Authors' own elaboration)

dieting students (89.8%) had iron deficiency and were classified as IDA subjects.

Meanwhile, only 6.1% of the anemic dieters had VB12 deficiency, and the same low proportion was reported of anemic subjects with folate deficiency. In addition, Chi-square test was employed to assess the degrees of dependency of anemia development on the presence of the three variables, demonstrating that occurrence of anemia among dieting participants is significantly dependent on iron deficiency (p<0.001). However, the findings revealed that anemia has developed among dieting students independently of VB12 or folate deficits (p>0.05).

Effect of BMI on Anemia and Iron Deficiency Among Dieting Participants

The comparison of Hb and ferritin concentrations among subgroups of dieting students (healthy BMI vs. over-weight vs. obese) found significant variations in Hb levels across dieting participants based on their BMI categories (p=0.019) (**Figure 3**). Dieting female participants with a healthy BMI had the lowest mean Hb concentration (11.3 g/dL), while over-weight and obese dieters had 12.3 and 12.2 g/dL, respectively. In addition, as compared to over-weight (21.0 ng/mL) and obese (18.6 ng/mL) individuals, the healthy BMI dieting group had the lowest mean ferritin levels (13.4 ng/mL). However, the change in ferritin levels was not statistically significant, as demonstrated by a p-value of 0.104.

Furthermore, the development of anemia in female WLDP participants was found to be significantly dependent on their BMI category (p=0.007), with dieter students of healthy BMI having the highest proportion (65.9%) of anemic subjects, followed by obese (34.8%) and over-weight (34.1%) dieting participants (**Table 4**). Similarly, individuals who participated in WLDPs despite having a healthy BMI had the greatest prevalence of iron deficiency and IDA (78.0% and 61.0%, respectively). Meanwhile, overweight and obese dieters had lower percentages of iron deficient individuals (43.9% and 39.1%, respectively), as well as IDA (26.8%, and 30.4%, respectively). Furthermore, the Chi-square test confirmed the significance reliance of iron deficiency and IDA prevalence among dieting females on their BMI categories, with p-values equal to 0.001 and 0.004, respectively.

Effects of Eating Habits and Practiced WLDPs on Anemia Prevalence Among Dieting Female Students

The questionnaire data was processed to show the frequency and percentages of anemic and non-anemic respondents in each response to the questions asked (**Table 5**).

In addition, the statistical relationship between anemia development and various eating habits and WLDP practices was investigated using the Chi-square test. The results showed significant effects of the students' source of information regarding their adopted WLDPs on the risk of anemia development (p=0.021). The highest anemia prevalence (60.0%) was found among those who got their diet plan from relatives and friends, followed by websites (55.2%) and self-program (51.4%), while those who got their diet plan from a dietitian and other sources had lower proportions of anemic subjects (16.7% and 11.1%, respectively). Another factor influencing anemia development was the duration science attempting the WLDP, with increased time leading to a rise in the prevalence of anemia among participants (p=0.016). Furthermore, among dieting students, skipping meals was shown to be substantially linked with the development of

Table 4. Distribution of anemia, iron deficiency and IDA among BMI categories of dieting female students

		Healthy BMI n/41 (%)	Over-weight n/41 (%)	Obese n/23 (%)	p-value
Anemia	Positive	27 (65.9%)	14 (34.1%)	8 (34.8%)	0.007
	Negative	14 (34.1%)	27 (65.9%)	15 (65.2%)	
Iron deficiency	Positive	33 (80.5%)	18 (43.9%)	9 (39.1%)	<0.001
	Negative	8 (19.5%)	23 (56.1%)	14 (60.9%)	
IDA	Positive	26 (63.4%)	11 (26.8%)	7 (30.4%)	0.002
	Negative	15 (36.6%)	30 (73.2%)	16 (69.6%)	

Note. Data was expressed as n (% of n among BMI categories); Chi-square test was used to assess variables independence; & statistically significant association was considered at p<0.05

Table 5. Distribution of anemic and non-anemic subjects according to the dietary habits and the practices of dieting participants

Variables	Answers	Within dieting n/105 (%)	Anemia		p-value
			Positive	Negative	
The main aim of attempting WLDPs	Improve body image	54 (51.4%)	23 (42.6%)	31 (57.4%)	0.348
	Prevent diseases	42 (40.0%)	23 (54.8%)	19 (45.2%)	
	Facilitate mobility	9 (8.6%)	3 (33.3%)	6 (66.7%)	
Source of attempted WLDPs	Dietitian	12 (11.4%)	2 (16.7%)	10 (83.3%)	0.021
	Websites	29 (27.6%)	16 (55.2%)	13 (44.8%)	
	Friends and relatives	20 (19.0%)	12 (60.0%)	8 (40.0%)	
	Self-program	35 (33.3%)	18 (51.4%)	17 (48.6%)	
	Others	9 (8.6%)	1 (11.1%)	8 (88.9%)	
Score of daily exercising	Never-rarely	10 (9.5%)	4 (40.0%)	6 (60.0%)	0.194
	Seldom-occasionally	35 (33.3%)	11 (31.4%)	24 (68.6%)	
	Sometimes	33 (31.4%)	19 (57.6%)	14 (42.4%)	
	Often-frequently	17 (16.2%)	10 (58.8%)	7 (41.2%)	
	Usually-always	10 (9.5%)	5 (50.0%)	5 (50.0%)	
Duration since attempting the program	3-4 months	39 (37.1%)	9 (23.1%)	30 (76.9%)	0.016
	4-5 months	30 (28.6%)	16 (53.3%)	14 (46.7%)	
	5-6 months	22 (21.0%)	13 (59.1%)	9 (40.9%)	
	>6 months	14 (13.3%)	11 (78.6%)	3 (21.4%)	
Daily intake of pure water	<1 l	9 (8.6%)	3 (33.3%)	6 (66.7%)	0.795
	1-2 l	39 (37.1%)	17 (43.6%)	22 (56.4%)	
	2-3 l	33 (31.4%)	18 (54.5%)	15 (55.5%)	
	3-4 l	18 (17.1%)	8 (44.4%)	10 (55.6%)	
	>4 l	6 (5.7%)	3 (50.0%)	3 (50.5%)	
Counting of daily calories consumption	Never-rarely	47 (44.8%)	24 (51.1%)	23 (48.9%)	0.482
	Seldom-occasionally	23 (21.9%)	11 (47.8%)	12 (52.2%)	
	Sometimes	14 (13.3%)	7 (50.0%)	7 (50.0%)	
	Often-frequently	14 (13.3%)	6 (42.9%)	8 (57.1%)	
	Usually-always	7 (6.7%)	1 (14.3%)	6 (85.7%)	
Intake of medication or herbal products	Herbal products	18 (17%)	13 (72.2%)	5 (27.8%)	0.103
	Non-prescribed drugs	9 (8.6%)	4 (44.4%)	5 (55.6%)	
	Prescribed drugs	4 (3.8%)	1 (25.0%)	3 (75.0%)	
	Nothings	74 (70.5%)	31 (41.9%)	43 (58.1%)	
Skipped meals	Skipping breakfast only	22 (21.0%)	14 (63.6%)	8 (36.4%)	0.002
	Skipping lunch only	8 (7.6%)	1 (12.5%)	7 (87.5%)	
	Skipping dinner only	16 (15.2%)	4 (25.0%)	12 (75.0%)	
	Skipping breakfast & lunch	16 (15.2%)	10 (62.5%)	6 (37.5%)	
	Skipping breakfast & dinner	27 (25.7%)	17 (63.0%)	10 (37.0%)	
	Take all meals	16 (15.2%)	3 (18.7%)	13 (81.3%)	

Note. Data were expressed as n within dieting (%), n of anemic (%), and n of non-anemic (%); Chi-square test was used to assess variables independence; & statistically significant association was considered at p<0.05

anemia (p=0.002). The greatest anemia rate was identified among those who skipped breakfast (63.6%), those who skipped both breakfast and dinner (63.0%), and those who skipped both breakfast and lunch (62.5%). Meanwhile, skipping only dinner or lunch, as well as eating all regular meals, was associated to a lower rate of anemia development (25.0%, 12.5%, and 18.7%, respectively).

However, there was no significant link between anemia development and the other examined factors including the main aim of attempting WLDP (p=0.348), score of daily exercising (p=0.194), daily intake of pure water (p=0.795), counting of daily calories consumption (p=0.482), and taking of medication or herbal products (p=0.103).

Reported Symptoms Among Diet Female Students and Their Association with Anemia

Dieting participants were asked to answer a multiple-selection question on the symptoms they experienced after starting their WLDPs. A total of 79 (75.2%) dieting participants reported suffering one or more symptoms since beginning the diet. The most frequently noted condition was falling hair, which was indicated by 59 (56.2%) respondents, followed by fatigue (47.6%), irregular and painful period (44.8%), lack of concentration (43.8%), irritable mood (29.5%), painful extremities (12.4%), and recurrent infection (9.5%) (**Table 6**). In general, there was a substantial connection between anemia and identifying the existence of symptoms (p<0.001). A total of 47 responders (59.5%) of those who stated suffering from at least one condition had positive anemia results, accounting for 95.9% of anemic subjects. Meanwhile, only two (7.7%) of those who did not observe any symptoms were found to be anemic, accounting for only 4.1% of anemic participants. Specifically, anemia was significantly correlated with suffering of fatigue (88.0%), irregular and painful period (85.1%), lack of concentration (80.4%), and falling hair (67.8%), showing p<0.001. However, irritable mood, painful extremities, and recurrent infection were independent of anemia, showing p-values >0.05.

DISCUSSION

Many people, particularly women, are concerned about reducing weight in order to treat obesity and have a positive perception of themselves. Several WLDPs have been scientifically demonstrated to help people lose weight and minimize their risk of obesity-associated health disorders,

Table 6. Distribution of anemic and non-anemic participants according to the reported symptoms among dieting female students

Symptoms	Answers	Within dieting n/105 (%)	Anemia		p-value
			+	-	
Fatigue	Yes	50 (47.6%)	44 (88.0%)	6 (12.0%)	<0.001
	No	55 (52.4%)	5 (9.1%)	50 (90.9%)	
Loss of concentration	Yes	46 (43.8%)	37 (80.4%)	9 (19.6%)	<0.001
	No	59 (56.2%)	12 (20.3%)	47 (79.7%)	
Irritable mood	Yes	31 (29.5%)	15 (48.4%)	16 (51.6%)	0.494
	No	74 (70.5%)	34 (45.9%)	40 (54.1%)	
Falling hair	Yes	59 (56.2%)	40 (67.8%)	19 (32.2%)	<0.001
	No	46 (43.8%)	9 (19.6%)	37 (80.4%)	
Painful extremities	Yes	13 (12.4%)	6 (46.2%)	7 (53.8%)	0.603
	No	92 (87.6%)	43 (46.7%)	49 (53.3%)	
Irregular & painful period	Yes	47 (44.8%)	40 (85.1%)	7 (14.9%)	<0.001
	No	58 (55.2%)	9 (15.5%)	49 (84.5%)	
Recurrences infections	Yes	10 (9.5%)	5 (50.0%)	5 (50.0%)	0.541
	No	95 (90.5%)	44 (46.3%)	51 (53.7%)	
Nothing	Yes	26 (24.8%)	2 (7.7%)	24 (92.3%)	<0.001
	No	79 (75.2%)	47 (59.5%)	32 (40.5%)	

Note. Data are expressed as n within dieting (%), n of anemic (%), and n of non-anemic (%); Chi-square test was used to assess variables independence; & statistically significant association was considered at p<0.05

while also ensuring adequate nutritional intake [3]. Attending a restricted WLDPs without careful supervision, on the other hand, can result in nutritional deficiency and changes in body compositions, leading to the development of a variety of physiological and psychological disorders [15, 33]. The purpose of this study was to investigate the influence of WLDPs performed by female university students on health parameters including anemia profile, serum levels of micronutrient, and biochemical markers of organ dysfunction, as well as the association of the detected abnormalities with diet habits of WLDPs participants. The study focused on female students based on the results of our previous survey, which revealed that 91.5% of dieting students are females, and since females are more prone to certain nutritional disorders, such as IDA, than males [25, 28].

Biochemical analysis indicated that there were no significant differences between the dieting and control groups in serum markers of liver function, renal function, and trace elements copper and zinc. This demonstrated the safety of the attempted WLDPs in this aspect. However, the main issue with female students following WLDPs was a rise in the rate of anemia, as evidenced by analysis of hematological indices as well as ferritin and VB12 levels. The dieting group showed significantly more anemic subjects with lower Hb concentration, serum ferritin and VB12 levels. In addition, dieter participants exhibited lower levels of PCV, MCV, and MCH, accompanied with higher platelets count and RDW value.

WHO defines anemia as having >13 g Hb/dL for men and >12 g Hb/dL for women [30]. Anemia is one of the most significant worldwide disorders affecting almost a third of the world population [24]. Due to menstruation, women of reproductive age are at a high risk of having anemia [34]. Findings of the present study in agreement with several previous reports, which have acknowledged a high prevalence of overall anemia among Jordanian adult females that raised from 26% in 2002 to 43% in 2017 reports [26, 35-37].

Anemia could be occurred due to wide range of factors, including nutritional factors such as VB12, folate, and iron deficiencies, as well as non-nutritional causes such as kidney dysfunction, chronic inflammatory disorders, and genetic traits like thalassemia and sickle cell anemia [38]. However, the current study focused on nutritional anemia as it could be a consequence of unhealthy diet regimen, and because only participants who did not have any known health problems were included in the study.

Assessment of the statistical dependency of anemia development on the deficits of the essential nutritional factors including iron, VB12, and folate, revealed a significant role of iron deficiency in the development of anemia among WLDPs group, accounting for 89.8% of anemic dieters. These findings are, in part, consistent with a previous report, which found that IDA was present in a significant proportion (63.3%) of anemic women of reproductive age [27].

The higher IDA proportion demonstrated in our results than the previous report could be attributed to practicing unhealthy WLDPs by the participants of the current research, causing insufficient consumption of iron dietary sources. It is well known that IDA arises when the iron intake is inadequate to meet the basic requirements for Hb synthesis and RBC production [39]. IDA is associated with hypochromic and microcytic RBCs indices, thrombocytosis and elevated RDW value [40]. This comes in line with our results, which showed that anemic dieting participants had significantly lower PCV, MCV, and MCH levels, as well as higher PLTs count and RDW compared with non-dieting participants.

Anemia and IDA were shown to be more prevalent among dieting students with a healthy BMI. This might point out to the involvement of body image misperception, despite having a healthy BMI, in female students' decision to practice unhealthy WLDPs. It has been demonstrated that young women frequently misperceive their physical body shape and weight, which may contribute to alterations in weight-related health behaviors as well as the development of eating disorders [41, 42]. Body size misperceptions in young adult females were found to be positively correlated with the development of eating disorder symptoms such as dieting, excessively restricting, and limiting the quantity and types of food consumed, denying feeling hungry, and avoiding certain foods [14]. This unhealthy dietary habit could result in deficiency of various essential nutrients as well as the development of nutritional diseases such as anemia, particularly IDA [43]. Accordingly, an inverse correlation was found between anemia development and women BMI values in Jordan [44].

Statistical analysis for the prevalence of anemia among dieting students based on the characteristics of the attempted WLDPs and dietary habits revealed a significant relationship between the risk of anemia development and the source of information about the tried diet programs, the length of time participants have attended the program, and the habit of skipping meals. Aside from professional-guided WLDPs, dieters can learn about WLDPs via books, websites, friends, and relatives, or by trying a self-proposed dietary plan [45]. The optimal WLDP should be successful in terms of weight reduction and long-term weight management, as well as safe, healthful, and ensuring adequate nutrient intake [3]. The efficacy and safety of WLDPs vary greatly depending on several factors such as dieter sex, developmental stage, health state, genetic profile, and the ratios of body composition, hence, there is no "gold standard" prescribed dietary plan that is appropriate for everyone [46, 47]. Therefore, random selection of WLDP without taking these criteria into account might explain the considerably greater anemia frequencies among participants of this study who rely on non-professional sources of diet plans such as websites, friends and relatives, and self-settled programs. Research has shown that development of dietary restriction-induced IDA depends on the severity of dietary restriction, the phase of growth and puberty during which shortage of iron intakes occurs, and the duration of dietary restriction [48, 49]. In this context, our data revealed a positive relationship between the prevalence of anemia, particularly IDA, among young adult women and the length of time since beginning the WLDPs. The habit of skipping breakfast, either alone or with any other major meal, was also observed to substantially increase the risk of anemia development among dieting participants. Likewise, previous studies have linked the rise in anemia prevalence among female high school and university students to dietary changes, specifically skipping breakfast [50, 51]. Breakfast intake has been found to be important in terms of nutritional balance. The regular intake of breakfast enhanced micronutrients consumption and improved the overall diet quality, while the habit of skipping breakfast by adolescents and adults was linked with health-risking practices [52, 53]. Furthermore, the consumption of high-quality breakfasts was found to considerably boost iron and other important micronutrient intake [54].

The present study demonstrated various self-reported symptoms including falling hair, fatigue, irregular and painful period, loss of concentration, irritable mood, painful extremities, and recurrent infection, which have been observed by the dieting participants after practicing the WLDPs. Also, anemia is often accompanied by a variety of symptoms, including fatigue, irritability, weakness, hair loss, and impaired concentration, which are physiologic reactions to a shortage of oxygen supply to tissues [55, 56]. Accordingly, we hypothesized that anemia was the main cause of reported symptoms among dieting individuals. As a result, the statistical analysis revealed a substantial association between anemia and fatigue, irregular and painful menstruation, loss of concentration, and falling hair indicating that anemia, specifically IDA, was the reason behind the most frequent symptoms arisen in dieting females.

In conclusion, practicing WLDPs without expert supervision increases the risk of nutritional deficiencies and the development of IDA in young females. Young girls with healthy BMI are the most vulnerable to constrained diet-induced nutritional health disorders like IDA. One of the unhealthiest dietary habits associated with the development of IDA in dieting young girls is skipping breakfast. Furthermore, nutritional anemia, primarily IDA, is responsible for a wide spectrum of physical and cognitive symptoms in females who attempt unhealthy WLDPs. Finally, it is strongly recommended to launch an education program to advise young females on when and how to adopt healthy WLDPs that ensure effective and safe weight loss while also verifying enough nutritional intake.

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Declaration of interest: No conflict of interest is declared by authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

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APPENDIX A: SUPPLEMENTARY TABLES

Table A1. Distribution of anemia, iron deficiency, IDA, VB12 deficiency, and folate deficiency among dieting and non-dieting female students

		Within dieting n/105 (%)	Within non-dieting n/80 (%)	Within total n/185 (%)	P-value
Anemia	Positive	49 (46.7%)	23 (28.7%)	72 (38.7)	0.013
	Negative	56 (53.3%)	57 (71.3%)	113 (61.1%)	
Iron deficiency	Positive	60 (57.1%)	27 (33.8%)	87 (47%)	0.002
	Negative	45 (42.9%)	53 (66.3%)	98 (53.0%)	
IDA	Positive	44 (41.9%)	12 (15.0%)	56 (30.3%)	<0.001
	Negative	61 (58.1%)	68 (85.0%)	129 (69.7%)	
VB12 deficiency	Positive	10 (9.5%)	3 (3.8%)	13 (7.0%)	0.128
	Negative	95 (90.5%)	77 (96.3%)	172 (93.0%)	
Folate deficiency	Positive	6 (5.7%)	4 (5.0%)	10 (5.4%)	0.83
	Negative	99 (94.3%)	76 (95.0%)	175 (94.6%)	

Note. Data are expressed as number (n) (% of n among dieting or non-dieting groups); Chi-square test was used to detect variables independence; & statistically significant association was considered at p<0.05

Table A2. Distribution of iron, VB12, and folate deficiencies among anemic and non-anemic dieting female students

Disorders	Results	Anemic n/49 (%)	Non-anemic n/56 (%)	p-value
Iron deficiency	Positive	44 (89.8%)	16 (28.6%)	<0.001
	Negative	5 (10.2 %)	40 (71.1%)	
VB12 deficiency	Positive	3 (6.1%)	7 (12.5%)	0.220
	Negative	46 (93.9%)	49 (87.5%)	
Folate deficiency	Positive	3 (6.1%)	3 (5.4%)	0.595
	Negative	46 (93.9)	53 (94.6%)	

Note. Data are expressed as number (n) (% of n among dieting or non-dieting groups); Chi-square test was used to detect variables independence; & statistically significant association was considered at p<0.05