Original Article

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Evaluation of Iron Deficiency and the Intake of Macro- and Micronutrients among Normal, Overweight, and Obese Children Under 5 Years in Amman

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Abstract

Background: This study primarily aimed to investigate the possible association between the risk of suffering from Iron Deficiency (ID) and body weight status among a group of obese, overweight, and normal body weight children. The second aim of this study was to assess Serum Iron (SI), Serum Ferritin (SF), Total Iron Binding Capacity (TIBC), Hemoglobin (Hb), and Body Mass Index (BMI) among the recruited children.

Materials and Methods: In this case-control study, a total of 150 disease-free children aged between 6-59 months were recruited conveniently from Amman. Children were grouped as normal body weight, overweight, and obese. BMI and BMI Z-scores were determined and the intake of many macro- and micronutrients were estimated. Serum iron, SF, TIBC, and Hb were measured to detect the presence of ID.

Results: The mean concentration of SI was significantly higher in normal body weight than in overweight and obese children. The mean concentration of TIBC was significantly lower in normal body weight children than that in overweight and obese children ($P<0.05$). Nutrients intake findings showed that daily intakes of saturated fat and sugar were significantly higher in overweight and obese children than those in normal children. Iron intake was significantly lower in normal body weight than in obese children. The daily intake of selenium was significantly higher among normal body weight and obese children than overweight children ($p<0.05$).

Conclusion: This study supports the findings of the presence of an association between weight gain and ID despite that iron intake among obese children was higher than those with normal body weight.

Keywords: Iron Deficiency, Obesity, Overweight, Macronutrients, Micronutrients

Introduction

Iron deficiency (ID) is the most common nutritional deficiency in children especially infants and toddlers (1), which could be related to their rapid growth and increased iron need as compared to older children (1). ID is a nutritional deficit that occurs due to inadequate levels of iron in the body (2). The World Health Organization (WHO) estimates that iron deficiency anemia (IDA) affects one quarter of the world's population and is concentrated within preschool-aged children and women; a majority of the anemia is due to ID. The negative consequences of IDA on cognitive and physical development of children, and work productivity of adults are of major concern (3). ID affects growth and development during early life; both are associated with impaired cognitive abilities (4). Additionally, Fallah et al., (2013) demonstrated that iron deficiency can be an important risk factor for development of febrile convulsion (5).

Overweight and obesity are major risk factors for chronic illnesses such as cardiovascular diseases. Children with overweight or obesity are also at high risk of non-communicable diseases (NCDs), premature death and disability in adulthood (6). In addition, the economic costs of escalating problem of childhood overweight and obesity are considerable, both in terms of the enormous financial strains which places on health-care systems and in terms of lost economic productivity (7). The incredible increase in

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obesity prevalence among children and adolescents could be attributed to the time spent in sedentary leisure activities such as watching television, playing video games, and using computers because of technology progress (8). Moreover, the increase in the consumption of foods high in energy density and low in micronutrients, especially iron, lead to the rise in obesity and ID occurrence among children and adolescents (9). The prevalence of ID in the United States is much higher among obese toddlers reaching a level of 20.3%, while for overweight and normal weight toddlers it was 8.1% and 7.1% respectively (2). However, in Jordan, the prevalence of overweight between children almost doubled between 1997 (4%) and 2009 (7%) according to Jordan Population and Family Health Survey (JPFHS) in 1997, 2002, 2009, while the prevalence of anemia among Jordanian children aged 6-59 months was about (32-34%) and remained stable during the period of 2009-2013 (10).

To our knowledge, there are no studies that focused on the association between body weight status and ID among children aged below 5 years in Jordan. Furthermore, the increase in the prevalence of ID as well as obesity among Jordanian children is alarming and more attention should be paid to this health problem. Therefore, the objectives of this study were to assess the levels of serum iron, serum ferritin, total iron binding capacity and hemoglobin in a selected group of sample of obese, overweight and normal body weight children; to evaluate nutrients' intake (macro- and micronutrients particularly iron intake) for the selected children by using 3 days' food records; to investigate the correlation between the children nutrients' intake (of some macro- and micronutrients) and their body mass index (BMI) and the obtained biochemical parameters; and to investigate the association between body weight status and the risk of suffering from ID among the recruited children.

Materials and Methods

Design and Study Sample

A case-control study was carried out during July 2016 to April 2017 to evaluate ID among overweight and obese children above 6 months and below 5 years, in a group of Jordanian children in Amman, Jordan. Infants below 6 months were excluded because most of them were breastfed and it was difficult to estimate precisely the nutrients intake. In this study, BMI and BMI Z-scores were calculated and the intake of many macro- and micronutrients were estimated. Serum iron (SI), serum ferritin (SF), total iron binding capacity (TIBC), and hemoglobin (Hb) were measured to detect the presence of ID. In this study, ID was defined using TIBC cut-off values which equal 240-450(μg/dl). A total of 175 children were assessed from Jordan University Hospital (Pediatric clinic) and from three Maternal and Child Centers of Ministry of Health for eligibility to participate in this study. Twenty-five participants were excluded because of their withdrawal due to blood sampling or incomplete dietary assessment. A total of 150 apparently healthy male and female children aged between 6-59 months were recruited conveniently according to inclusion and exclusion criteria and their medical history. The participants were divided into three groups of 50 obese, 50 overweight and 50 normal weight children depending on the BMI values. Children recruitment was carried out under the supervision of pediatrician. Inclusion criteria were Jordanian with normal body weight, overweight or obese boys or girls and 6 to less than 59 months old. Children who were suffering from known case of ID anemia, celiac, rheumatoid, liver, pulmonary, or kidney diseases, carcinoma, thalassemia, intestinal parasitic infections, physical disability, endocrine disorders and neurological illness, taking

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supplements containing iron and being on special therapeutic diet were excluded from the study. Parents of eligible children were asked to participate in the study. Parents were provided with an informational form containing a brief description of the nature of the study. Then written consent form was obtained from parents. Furthermore, parents have been told that all children's results will be kept strictly confidential. Parents of children with ID were informed about their children ID status after collecting all the required information including dietary habits and medical history to make sure that no changes will take place until documenting the required information. The study was approved by the IRB committee of Jordan University Hospital and Ministry of Health. The ethical code for the IRB of Jordan University Hospital was 2016/131, while of the MOH was 2016/3741.

Anthropometric Measurements

Anthropometric measurements, including length, height and weight were determined according to Lee and Neiman (2013). Recumbent length (for children under age 24 months) was measured using calibrated length board while height (for children age 24 months and older) was measured without shoes using a calibrated Harpenden portable stadiometer. All the measurements were taken in duplicate with the children wearing light clothing and no shoes. Length and height was measured to the nearest 0.1 cm; and weight was measured to the nearest 0.1 kg on a portable Seca scale (model 0213) (Seca, Hammer Steindamm, Hamburg, Germany). BMI was calculated as: weight (kg)/ height (m)2. Overweight and obesity were categorized according to weight-for-age (z-score), according to WHO child growth standards (z- scores) (WHO, 2006) which defined overweight and obesity as ≥2SD and ≥ 3SD, respectively, and normal weight between 0 SD and less than 2 SD (12).

Blood Samples Collection and Biochemical Analyses

SI, SF, Hb, and TIBC were measured to find out children with ID. All parents kindly were asked to donate 5 ml venous blood sample from their children. The blood sample was drawn by a pediatric nurse at the nurse room. Tubes were labeled and transported in an ice box within an hour and analyzed at a private laboratory to check for SI, TIBC, and Hb according to the following biochemistry methods.

Non-hemolyzed serum samples were collected in the morning in order to avoid low results due to diurnal variation. Blood samples centrifuged at room temperature for 5 min at 6500xg. SF levels were examined using commercially available ELISA kit (Tina-quant Ferritin Gen. 4 Cobas c system, Ref. No. 04885317 190, Roche, Germany). Hemoglobin (Hb), SI, and TIBC were also analyzed in duplicate. Hemoglobin concentration was estimated by Colorimetric techniques based on light intensity principle. SI and TIBC were determined by quantitative calorimetric analysis, using commercial kit (Iron Gen.2 Cobas Integra/Cobas C System Ref. No.031836922 Roche, Germany) for measuring serum iron.

Cut-Off Values for Biochemical Iron Markers

Internationally accepted cut-off values for biochemical iron markers used in this study were as follows: Hb (g/l)= 9.5-14.5; SF (ng/ml) =29-160; and SI (µg/dl)= 25-115 (13).

Dietary Assessment

"Twenty-four- hour record" method was used for a duration of 3 days using the surrogate techniques to evaluate the daily intake of macronutrients, micronutrients and iron. Mothers were asked to record all foods and beverages (including snacks) at the time of eating during the food record period (2 random days and one weekend) of the food intakes (11). Standard

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measuring tools were used to help mothers estimate portion size. After completing the records, subject's dietary intakes were also analyzed using dietary analysis software (ESHA Food Processor SQL version 10.1.1; ESHA, Salem, OR) with additional data on foods consumed in Jordan (14).

Statistical Analysis

The data were statistically analyzed using SPSS (version 20). Categorical variables were analyzed by Chi square and reported as frequencies and percentages; whereas, continuous variables were presented as mean ± standard error. Post-hoc and Least Significant Difference (LSD) tests were used for multiple comparisons. Analysis of Variance (ANOVA) was used to determine differences of biochemical parameters (hemoglobin, iron serum, TIBC and ferritin), nutrients intake and BMI status among children which was stratified to three groups according to BMI status (normal weight, overweight and obese). The correlation between hemoglobin (g/l), iron serum, TIBC, ferritin serum levels with body mass index and different nutrients intake was tested by Pearson test (r). Linear Regression was performed to examine the association of being overweight or obese and the risk of having ID after adjusting for age. The risk was reported as odd ratio (OR) with 95% confidence interval (CI). Values at P-values < 0.05 was considered statistically significant.

Results

General Characteristic of the Sample Population

Table I presents the demographic and lifestyle characteristics of the sample of Jordanian children aged from 6 to 59 months based on their BMI status. For participant's age (months), duration of breast feeding (months), and frequency of milk consumption per day, a significant statistical difference (*P*-value <0.01) was detected between normal weight, overweight and obese children, with normal weight children showing oldest age, longest breast feeding duration, and least frequent to consume milk per day as compared to the other two groups. Amount of meat intake per day was found to be significantly (P-value=0.04) higher in obese body weight than overweight and normal children. On the other hand, the dairy intake was significantly (*P*=0.02) higher in overweight (1.6 ± 0.2) and obese (1.4±0.2) than in normal body weight children (0.9±0.2). Moreover, protein intake was significantly (*P*=0.001) higher in normal body weight children (2.2±0.2) than overweight (1.2±0.1) and obese weight children (1.5± 0.2). No significant difference was observed between children of three different groups of BMI status in the rest of variables.

Biochemical Measurements of Participants

The study findings illustrated in Table II revealed that the mean of hemoglobin is significantly (*P*=0.01) higher (12.2±0.2 g/l) in normal body weight and obese (12.0±0.1 g/l) children as compared with overweight children (11.6±0.2). The mean concentration of SI (µg/dl) was significantly (*P*=0.01) higher (67.1±4.0) in normal body weight children than in overweight and obese children (54.8±4.1 and 52.7±2.6, respectively). On the other hand, the mean concentration of TIBC (µg/dl) was significantly (*P*=0.02) lower (363.3±7.8) in normal body weight children than that in overweight and obese children (393.3±9.5 and 398.8±10.5, respectively). No significant differences were found among children in the three groups concerning mean concentration of ferritin.

Daily Nutrients Intake of Participants

Table III shows that the means of daily intakes of saturated fat (g) and sugar (g) were significantly higher in overweight and obese children than those in normal children. No significant difference was found between body weight status and daily total calories intake and daily calories that

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come from fat, except for calories from saturated fat which was significantly (P=0.01) higher in overweight (149.1±7.3 Kcal) and obese (139.4±7.2 Kcal) than in normal body weight children (117.1±6.5 Kcal). On the other hand, daily folate intake was significantly (P<0.001) lower in overweight (105.8±6.0 µg) and normal body weight (119.6±7.2 µg) than that in obese children (143.0 ±8.0µg). Similarly, the daily intake of iron was significantly (P=0.01) lower in normal body weight (16.3±1.6 mg) than in obese (23.6.0±23.6.0 mg) children. The daily intake of selenium was significantly (P=0.02) higher among normal body weight and obese children (18.7±2.3 µg and 16.8±2.1 µg, receptively) than overweight children (10.9±1.7µg). No significant differences were detected among normal body weight, overweight, and obese children regarding daily intake of other nutrients.

Correlations among Biochemical Indicators and Different Nutrients Intakes

In order to examine the association between hemoglobin, SI, TIBC, SF levels with BMI and different nutrients intake, partial correlations (r) were performed and the results are summarized in Table IV. The study findings revealed that BMI was positively and significantly correlated with, protein, carbohydrate, sugar, iron, vitamin C, and calories intake. While Hb was significantly correlated with vitamin (vitamin B6) and three minerals (calcium, selenium and iron), IS was positively and significantly correlated only with vitamin E-α-tocopherol and vitamin C. Additionally, TIBC was positively and significantly correlated with the intake of vitamin B_{12}. However, all the correlations were weak and not more than 0.35.

Association between BMI and Iron Deficiency:

Table V shows the OR (95% CI) of the risk of suffering from ID among overweight and obese weight children as compared to normal body weight children for the dependent variable TIBC level. The results revealed that overweight children had 4.5 times the risk of ID compared with normal body weight children. However, the obese children had 3.7 times the risk of ID compared with normal weight children, (P-value = 0.01).

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Table I: General and lifestyle characteristics of participants.

Variable	BMI Category			
	Mean ±SEM			
	Control (n=50)	Overweight (n=50)	Obese (n=50)	*P*-value
Age (months)	32.4±2.1[a]	18.6±1.6[b]	21.7±2.3[b]	**0.01**
Duration of Breast Feeding (months)	16.6±0.9[a]	11.6±0.9[b]	10.8±1.1[b]	**0.01**
Milk Frequency / day	2.1 ± 0.3[a]	4.0 ± 0.6[b]	3.7 ± 0.3[b]	**0.01**
Milk Amount Per Feeding (ml)	138.1 ± 8.9	135.9 ± 10.49	163.2 ± 6.7	0.06
Daily milk intake(ml)	313.9 ± 42.7[a]	556.1 ± 57.9[b]	608.6 ± 51.7[b]	**0.01**
Amount of Meat Intake (gm)	25.8 ± 3.2[a]	28.4 ± 3.3[a,b]	36.8 ± 2.9[a]	**0.04**
Grain (oz-e)	1.8 ± 0.2	1.5 ± 0.2	1.9 ± 0.2	0.30
Vegetables (cup)	0.5 ± 0.07	0.3 ± 0.1	0.5 ± 0.1	0.16
Fruit (cup)	0.9 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.08
Dairy Products (cup)	0.9 ± 0.2[a]	1.6 ± 0.2[b]	1.4 ± 0.2[b]	**0.02**
Protein (oz-e)	2.2 ± 0.2[b]	1.2 ± 0.1[a]	1.5 ± 0.2[a]	**0.01**
Variables	**n (%)**	**n (%)**	**n (%)**	
Gender				
Male	30(60)	30 (60)	29 (58)	0.97
Female	20(40)	20 (40)	21 (42)	
Father Educational level				
Secondary and below	31 (62)	33 (66)	31 (62)	0.18
Diploma	10 (20)	6 (12)	11 (22)	
BSc	9 (18)	8 (16)	8 (16)	
MSc	0 (0)	3 (6)	0 (0)	
Mother occupation Status				
Housewife	41 (82)	38 (76)	38 (79)	0.76
Employee	9 (18)	12 (24)	12 (21)	
Feeding Type from Birth				
Breastfeed	46 (92)	47 (94)	49 (98)	0.39
Bottle-feed	4 (8)	3 (6)	1 (2)	
Neonatal Unit. Duration (days)				
1-4	44 (88)	47 (94)	50 (100)	0.15
5-8	4 (8)	1 (2)	0 (0)	
9-14	2 (4)	2 (4)	0 (0)	
Child Inactivity				
YES	2 (4)	1 (2)	2 (4)	0.81
NO	48 (96)	49 (98)	48 (96)	
Nursery				
YES	4 (8)	5 (10)	7 (14)	0.56
NO	46 (92)	45 (90)	43 (86)	
ID. Based on IS				
Deficiency	17 (34)	18 (36)	8 (16)	0.51
Normal	33 (66)	32 (64)	42 (84)	
ID Based on TIBC				
Deficiency	5 (10)	12 (24)	20 (40)	.074
Normal	45 (90)	37 (76)	30 (60)	

Data are presented as the mean ± SEM and as percentages; all are considered statistically significant at P< 0.05. Means within the same row with different superscript letters are significantly different. Abbreviations: SI: Serum Iron and TIBC: Total Iron Binding Capacity.

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Table II: Biochemical measurements of participants

Variable	BMI Categories (Mean± SEM)			
	Normal (n=50)	Overweight (n=50)	Obese (n=50)	*P*-value
Hemoglobin (g/l)	12.2±0.2[a]	11.6±0.2[b]	12.0±0.1[a]	**0.01**
SI (µg/dl)	67.1±4.0[a]	54.8±4.1[b]	52.7±2.6[b]	**0.01**
TIBC(µg/dl)	363.3 ±7.8[a]	393.3 ±9.5[b]	398.8 ±10.5[b]	**0.02**
SF(ng/ml)	20.4 ±2.6	23.1±3.0	21.2 ±2.5	0.77

- Data are presented as the mean ± SEM and as percentages; all are considered statistically significant at P< 0.05.
- Abbreviations: SI: Serum Iron, SF: Serum ferritin and TIBC: Total Iron Binding Capacity.

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Table III: Nutrient intakes of participants according to the BMI categories.

Variable	BMI categories (Mean± SEM)			*P*- value
	Normal (N=50)	Overweight (N=50)	Obese (N=50)	
Calories (kcal)	961.9±32.5	936.7±33.0	976.8±30.3	0.67
Calories from Fat (kcal)	363.9±16.2	380.7±12.0	363.3±11.2	0.58
Fat percent from total Kcal. (%)	37.8	40.6	37.2	
Calories from saturated fat (kcal)	117.1±6.5[a]	149.1±7.3[b]	139.4±7.2[b]	
Saturated fat percent from total Kcal. (%)	12.2[a]	15.9[b]	14.3[b]	**0.01**
Saturated Fatty acid (g)	13.4±0.8[a]	17.1±0.8[b]	15.9±0.9[b]	
Protein (g)	35.7±1.6	33.3±1.7	37.4±1.3	
Protein percent from total Kcal. (%)	14.8	14.2	15.3	0.14
Carbohydrate (g)	115±4.3	105.5±4.8	118.9±4.9	
Carbohydrate percent from total Kcal. (%)	47.8	45	48.7	0.11
Fiber (g)	6.1±.4	4.6±0.5	5.7±0.5	0.06
Sugar (g)	49.7±2.3[a]	58.1±2.3[b]	57.2±3.0[b]	**0.04**
Fat (g)	40.3±1.8	42.4±1.3	40.5±1.3	0.54
Vitamin B6 (mg)	0.4±0.1	0.4±0.1	0.5±0.1	0.10
Vitamin B12 (µg)	2.5±0.5	2.6±0.4	3.2±0.3	0.47
Vitamin C (mg)	28.4±2.7	33.3±2.6	37.2±3.8	0.11
Vitamin E-α-Toco (mg)	1.4±0.2	1.4±0.1	1.6±0.1	0.51
Folate (µg)	119.6±7.2[a]	105.8±6.0[a]	143.1±8.1[b]	**0.01**
Calcium (mg)	236.6±35.2	173.9±38.5	286.6±51.9	0.18
Copper (mg)	2.1±0.3	2.5±0.3	2.1±0.4	0.60
Iron (mg)	16.3±1.6[a]	20.3±1.8[a,b]	23.6±23.6[b]	**0.01**
Selenium (µg)	18.7±2.3[a]	10.9±1.7[b]	16.8±2.1[a]	**0.02**
Zinc (mg)	11.1±2.6	16.4±3.8	8.6±1.4	0.12

- Data are presented as the mean ± SEM; and are considered statistically significant at $P<0.05$.
- Means within the same row with different superscript letters are significantly different.

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Table IV. Correlations among biochemical indicators and different nutrients intakes

Indicators*		BMI	Hb	SI	TIBC	SF
BMI	r. Pearson		0.08	-0.19	0.03	0.12
	P- value		0.52	0.11	0.80	0.29
Calories (Kcal)	r. Pearson	**0.23**	0.05	0.05	-0.05	0.17
	P- value	**0.04**	0.66	0.64	0.65	0.14
Calories from Fat (kcal)	r. Pearson	0.15	-0.06	0.01	0.09	-0.02
	P- value	0.19	0.58	0.94	0.45	0.85
Calories from Saturated fat (kcal)	r. Pearson	0.20	-0.19	0.04	0.22	-0.16
	P- value	0.08	0.10	0.73	0.05	0.17
Protein (g)	r. Pearson	**0.25**	0.22	0.08	0.18	-0.03
	P- value	**0.03**	0.06	0.47	0.12	0.83
Carbohydrate (g)	r. Pearson	**0.24**	0.08	0.04	-0.16	0.22
	P- value	**0.04**	0.48	0.73	0.16	0.06
Fiber (g)	r. Pearson	0.10	0.03	0.15	-0.18	0.19
	P- value	0.40	0.81	0.20	0.13	0.10
Sugar (g	r. Pearson	**0.28**	-0.18	-0.01	-0.18	0.04
	P- value	**0.01**	0.12	0.91	0.12	0.72
Fat (g)	r. Pearson	0.15	-0.06	0.01	0.09	-0.02
	P- value	0.20	0.62	0.93	0.45	0.86
Saturated Fat (g)	r. Pearson	0.20	-0.19	0.04	0.22	-0.16
	P- value	0.08	0.10	0.73	0.05	0.17
Vitamin B6 (mg)	r. Pearson	0.04	**0.26**	0.09	0.06	-0.01
	P- value	0.74	**0.02**	0.44	0.62	0.91
Vitamin B12 (µg)	r. Pearson	0.07	0.17	0.22	**0.25**	0.00
	P- value	0.53	0.13	0.06	**0.03**	0.97
Vitamin C (mg)	r. Pearson	**0.29**	-0.22	**0.27**	0.02	-0.04
	P- value	**0.01**	0.05	**0.02**	0.88	0.75
Vitamin E-α-Toco (mg)	r. Pearson	0.12	0.08	**0.31**	0.06	-0.07
	P- value	0.30	0.49	**0.01**	0.62	0.54
Folate (µg)	r. Pearson	-0.17	0.06	-0.01	0.05	-0.11
	P- value	0.15	0.64	0.95	0.20	0.35
Calcium (mg)	r. Pearson	0.09	**0.32**	0.20	0.07	0.02
	P- value	0.43	**0.01**	0.08	0.56	0.66
Copper (mg)	r. Pearson	-0.01	-0.14	-0.12	-0.04	-0.05
	P- value	0.93	0.22	0.31	0.74	0.69
Iron (mg)	r. Pearson	**0.26**	**0.35**	0.05	0.23	0.00
	P- value	**0.02**	**0.00**	0.64	0.05	0.97
Selenium (µg)	r. Pearson	0.04	**0.34**	0.17	0.06	0.08
	P- value	0.72	**0.00**	0.15	0.61	0.51
Zinc (mg)	r. Pearson	0.01	-0.12	-0.06	0.06	0.07
	P- value	0.94	0.32	0.62	0.58	0.57

* Data are considered statistically significant at P<0.05.

- Abbreviations: BMI: Body Mass Index, Hb: Hemoglobin, SI: Serum Iron, SF: Serum ferritin and TIBC: Total Iron Binding Capacity.

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Table V: Odds ratios (95% confidence intervals) of overweight and obese weight children for association with iron deficiency based on TIBC[*]

	Normal	Overweight	Obese
		Iron deficiency Based on TIBC	
N of deficiency	3	11	9
N of Non deficiency	45	37	37
OR (95.CI)	1	4.5 (1.2-17.2)	3.7 (0.9-14.5)
***P*-value**		0.01	

* Data are considered statistically significant at P<0.05.

Values are age-adjusted correlation coefficients.

Discussion

Obesity is a low grade chronic proinflammatory disease that leads to imbalance between levels of iron biomarkers (15). The present study showed a significant negative correlation between the duration of BF and being overweight and obese (as duration of breastfeeding decreased, the possibility of being overweight and obesity increased). In other words, breastfed children for shorter durations were more likely to gain weight than breastfed infant for longer duration and it appears to be protective role against overweight and obesity in this selected sample of children. Similar result was obtained by Baker et al., (2014) who revealed that infants who are breastfed (≥20 weeks), gain less weight at 1 year than those who were formula fed or mixed-fed. Children who were breastfed (6 months or more) were less likely to be overweight and obese as compared to those who were never breastfed (17). Prolonged bottle-feeding was found to be significantly associated with both overweight and iron-deficiency in children 18 to 56 months of age (18).

A key finding of this study was the high occurrence of ID among overweight and obese children as compared to normal weight children. The present study showed a significant difference in ID variables (SI, Hb, TIBC) among overweight and obese children as compared to healthy ones. The risk of having ID is 4.5 and 3.7 odds among overweight and obese children, respectively, then normal weight children (P-value = 0.01). This finding agrees with results of Cepeda et al., (2010) who revealed that the risk of iron deficiency in Mexican obese children is 2-4 times higher than normal-weight children (4). However, the occurrence of ID in the present study based on TIBC was higher than the prevalence of ID reported for obese and overweight Swiss children which was (20% and 12%, respectively) (19). The frequency of ID reported by the present and the two studies cited above is noticeably higher compared to the result reported from the USA, specifically, the prevalence of ID reported for obese children in the Third National Health and Nutrition Examination Survey (NHANES) which was 2.4% and 9.1%, respectively (20). The analysis of hematological iron markers used in this study revealed significant differences in SI, TIBC, and Hb among the 3 groups of BMI; all of which are markers used to measure the level of circulating iron. These results are in agreement with the results reported by Sharif et al., (2014) who found that mean SI levels were lower among obese children in comparison with control group; however, ferritin concentrations were similar in both groups (21). The mean

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concentration of TIBC (ug/dl) was also significantly lower in normal body weight children than that in overweight and obese children. This is similar to results of Cepeda et al., (2010) and Nosrat et al., (2015) who found that the level of TIBC were higher in obese children than in normal body weight children (4, 22). By contrast, ferritin -based diagnosis of ID- is more likely to conclude that BMI is not associated with-or may even play a protective role in- the development of ID since there was no difference between groups in ferritin. In the present study, ferritin concentration was higher in overweight/obese children than those with normal body weight. The same result was reported by Hamza et al., (2013) who found that serum ferritin concentration was higher in overweight and obese Egyptian children, while other markers were indicative of a poorer iron status (23). The inflammatory state caused by obesity, may increase serum ferritin levels and higher levels of ferritin are a normal situation in obese people (21). Therefore, children with obesity-related ID may have normal or even elevated levels of serum ferritin; specifically, compared with non-overweight subjects. Obesity is associated with low-grade inflammation of White Adipose Tissue (WAT) due to chronic activation of the innate immune system (24). Both WAT and infiltrated macrophages can be a major source of inflammatory cytokines, such as tumors necrosis factor-α, which is an activator of ferritin transcription (25). Different factors have been proposed for the explanation of association between iron deficiency and obesity. These may include one or more of the following mechanisms. Firstly, the primary causes of ID include low intake of bioavailable iron; increased iron requirements as a result of rapid growth; excess blood loss caused by pathologic infections, such as hook worm and whipworm causing gastrointestinal blood loss (4), and impaired absorption of iron (26); secondly, inappropriate diet with intake of iron deficient foods and genetic factors (27, 20); thirdly, inflammation status related to the increased fat mass that mediates the suboptimal iron levels and consequently increase the risk for ID and anaemia (5); and finally, the combination of nutritional and functional factors (5). Another explanation of the association between iron deficiency and obesity is the role of hepcidin-mediated iron sequestration in obesity-related ID (28).

The results of the current study showed that calories intake and sugar are positively correlated with BMI. Many studies have examined the link between sugary drink consumption and weight and it has been continually found to be a contributing factor to become overweight (29-30). Furthermore, the Scientific Advisory Committee on Nutrition (31) reviewed randomized control trials, which indicated that consumption of sugars-sweetened beverages, as compared with non-calorically sweetened beverages, results in weight gain and an increase in BMI among children. Prospective cohort studies also generally confirmed the link between sugars-sweetened beverages and increased obesity (32). Another factor that contributes to childhood obesity is the consumption of empty calorie snack foods. Snack foods include foods such as chips, baked goods, and candy which provide high calories with few nutrients, including iron. While snacking has been shown to increase overall caloric intake, no studies have been able to find a link between snacking and overweight (29). The positive correlation detected in this study between the two major macronutrients (carbohydrates and protein) and BMI is similar to Grantham' et al., (2014) findings, who reported -in modern diet-carbohydrates are digested to satisfy body's energetic needs while protein is converted and stored as fat. In addition, carbohydrates are among the macronutrients that provide energy and can thus contribute to excess energy intake and subsequent weight gain (34).

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Regarding the protein intake, a study by Scaglioni et al., (2000) revealed that preschool overweight children had a higher percentage intake of proteins at the age of one year than non-overweight children (35). In our study, meat intake was found to be significantly associated with BMI. This finding is in agreement with the previous findings which indicate that high intakes of both red and processed meat have been shown to be positively related to weight gain (36). A recent study by Wenpeng et al., (2016) showed that meat availability is most highly correlated with prevalence of obesity and overweight and BMI (37). In addition, other studies mentioned that meat when consumed at high level, may increase weight gain due to its high energy density and/or fat content (38-39). The significant difference ($p<0.005$) between the study groups in the percent of energy intake from SFAs (12.5% to 16.4%) to the total energy intake is quite comparable with that reported by Rosa et al., (2001) who concluded that SFAs provided (15.2%) of the energy intake of the diet (40). Similar findings were documented by Niinikoski et al., (1997) who found that in 2-year-old children the percent of energy intake from SFAs was about 14.5% (41). Furthermore, the percent could reach 15% in 2- to 14-year-old Greek children (42). However, other studies indicated that transition towards a lower fat intake in preschool children should not be advised since this may be accompanied by reductions in energy and nutrient intakes (43, 44). Our data showed positive and significant correlations between vitamin B6 intake and hemoglobin. These results are in agreement with Leklem et al., (1999) who found that vitamin B6 helps in the synthesis of hemoglobin, by acting as a coenzyme for the enzyme Aminolevulinic Acid (ALA) synthase which is involved in the synthesis of heme, an iron-containing component of hemoglobin (45). Moreover, we found a positive correlation between hemoglobin and some minerals such as calcium, selenium and iron (45). In a previous study by Lind et al., (2004), a significant increase in Hb with increasing iron intake was reported, and the relation between dietary iron intake and Hb was linear (46). Miranda et al., (2014) found thatcombined calcium and iron is equally effective as single iron in reducing the prevalence of iron deficiency anemia in children (47).

Dalton et al., (1997) examined whether calcium of infant formula affects the healthy full-term infants after 4 to 9 months. They didn't find any differences between the experimental and control groups on Hb, SF, and TIBC (48). Lynch et al., (2000) reported that calcium has only a limited effect on iron absorption in a wide variety of foods and various concentrations of other inhibitors and enhancers (49). Absorption of iron is tightly regulated and affected by individual iron status more than dietary factors (50). In addition, calcium is an essential nutrient for normal growth and development, and requirements are higher during infancy and adolescence than in adulthood (51). A higher calcium intake may lead to the achievement of maximal bone mass (52-53). In the present study, obese children were found to consume more milk and calcium than did normal body weight children. This may help them in achieving a heavier weight and greater bone mass which may later protect them from diseases such as osteoporosis (53-54).

The significant correlation detected in the present study between selenium and Hb is in agreement with a study by Herbert et al., (1996) concluding that the concentrations of reactive oxygen species (ROS) are kept extremely low due to the activity of anti-oxidative enzyme systems, including glutathione peroxidases (GPx) (55). This enzyme contains selenium as a potent cofactor; therefore, an adequate daily intake of selenium may protect against harmful effects of ROS (55).

The present study results showed a significant association between vitamin E

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and vitamin C and SI. Herbert et al., (1996) highlighted that, in addition to adequate amounts of the anti-oxidative components vitamin C (ascorbate) and vitamin E, it should be noted that ferric complexes, react very slowly with H2O2 compared to ferrous forms of iron. Reducing agents can thereby stimulate the Fenton reaction and this may occur with simultaneous overdosing with vitamin C and iron: (Fe (III) + vitamin C> Fe (II) + Semi dehydroascorbate). Hence, whereas vitamin C in reasonable doses acts as an antioxidant, the same vitamin in mega doses (>1000 mg/day) may act as a prooxidant, especially in subjects with Fe overload (55). Reduction of oxidative stress by vitamin E may result in a significant increase in hemoglobin (56). On another hand, deficiency of vitamin E, especially in the infant, will lead to red blood cell rupture, and the magnitude of the anemia appears related to the presence of associated factors such as the lipid composition of the RBC membrane and the nature of associated oxidant stresses (57).

Regarding the relationship between the intake of iron and vitamin B12 and TIBC, we found a significant association between those two nutrients and TIBC. These findings are in agreement with the recent findings which indicate that, a reduced rate of iron intake represents a more advanced stage of iron depletion, which is associated with higher TIBC (58). On the other hand, iron and vitamin B12 deficiency, either singly or in combination, could be presented with not only disordered hematopoiesis, but also can precede the appearance of hematological abnormalities (59). The study had several limitations. First, blood iron status can be influenced by a variety of factors, including the contents of the meal consumed prior to sampling; although, other confounders were likely present. Second, though a significant association was detected between obesity and ID, no causal relationship can be inferred because of the merits of cross-sectional studies. Thus, longitudinal research is required in order to test the putative causal relationship between obesity and ID. Third, researchers in Jordan used to assess the dietary intake of Jordanians using an international food database (ESHA) that tracks the general market availability of different food types, not the actual and bioavailable consumption. There are no direct measures of actual human consumption that can account for food wastage and provide precise measures of food consumption nationally. These studies were generally limited in sample size. The sample in this study is not representative of the population as the children in this study were selected by convenience sampling from hospital, and three comprehensive health centers, and not from the general population, and we cannot generalize the results to the whole population. Therefore, a large scale study is warranted.

Conclusion

In conclusion, present study provided evidence supporting the presence of an association between obesity and ID. Weight gain measured by BMI is found to be a risk factor for developing ID. Different associations were found between the intake of some micronutrients (vitamins B6, C and E and calcium, iron, and selenium) and the measured ID blood biomarkers. Dietary assessment results revealed that the intake of saturated fat, sugar, and energy from saturated fat was significantly higher in overweight and obese children than that in normal children. While, the intake of folic acid and iron was higher in obese children as compared to normal body weight and overweight children, the intake of selenium was the lowest in overweight children as compared to the other two groups. TIBC is a promising candidate in detecting ID; although, the standardized assay system is still required. Overweight and obese individuals should undergo periodical screening for iron status, to

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prevent reaching to latent ID or even early stage of IDA.

Conflict of interest

The authors report no conflict of interest.

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