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Research Article

Effect of High-Fat-Diets on Iron Homeostasis and Tissue Iron Deposition in Female Sprague-Dawley Rats

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ABSTRACT

Background: Obesity has been associated with anemia of chronic disease and it may also promote iron deficiency causing dysmetabolic iron overload.

Objective: The aim of this study was to examine iron homeostasis on serum and tissue after feeding rats with various high-fat-diets for 6 and 10 weeks.

Methods: Eight weeks Sprague - Dawley female rats were randomly divided into three main dietary groups: Group (1): rats were fed the High Saturated Fat Diet Group (HSFD; n = 12), group (2): rats were fed the High Monounsaturated Fat Diet Group (HMUSFD; n = 12) and group (3): rats were fed Normal Fat Diet Group (NFD) (n = 12) for 6 and 10 weeks. Blood samples were collected; liver and Retroperitoneal Adipose Tissues (RPAT) was removed. Serum iron parameters and the total iron quantification in tissue were measured.

Results: Findings after 6 weeks demonstrated that no significant elevation in all tested iron parameters in serum and tissue in HFDs fed groups as compared to NFD controls. On the other hand, feeding rats with different HFDs for 10 weeks leading to increase serum ferritin significantly as compared to NFD, and liver iron content was increased significantly in rats that were fed HSFD and NFD (178.16 ± 7.78, 168.67 ± 5.42 ng/ mg, respectively) as compared to HMUFD (123.78 ± 6.57ng/ mg)($p < 0.05$).

Conclusion: The effect of the high-fat-diets on iron homeostasis is time dependent. However, the type of dietary fat that introduce will affect tissue iron deposition.

Keywords: High-fat-diets; Iron homeostasis; Tissue iron deposition

INTRODUCTION

Iron is not only an essential element for growth and survival, but also is a fundamental cofactor for several enzymes involved in oxidation–reduction reactions due to its ability to exist in two ionic forms: ferrous (Fe^{+2}) and ferric (Fe^{+3}) iron [1]. Obesity is a serious global health challenge with the pandemic proportion that results in a significant mortalities and morbidities [2]. It expands the white adipose tissue mass accompanied by adipose tissue remodeling and macrophages infiltration and inflammation [3].

Adipose tissue produces many cytokines and adipokines such as IL-6, interleukin-1β, interleukin-8, TNF-α, leptin, adiponectin, resistin, lipocalin-2, C-reactive protein (CRP), monocyte chemoattractant protein 1, complement components, plasminogen activator inhibitor-1 [4]. Moreover, adipose tissue of obese individuals expresses up regulation of pro-inflammatory cytokines and down-regulation of anti-inflammatory cytokines [5]; thus, leading to macrophages infiltration, ectopic fat accumulation, hypoxia and death of adipose tissue [6]. Additionally, obesity has been associated with anemia of chronic disease especially systematic iron deficiency and hypoferremia [5]. Obesity may also promotes iron deficiency by inhibition of dietary iron uptake from the duodenum causing Dysmetabolic Iron Overload Syndrome (DIOS), which is characterized by increased serum ferritin concentrations with normal or mildly elevated transferrin saturation in individuals with various components of metabolic syndrome or non alcoholic fatty liver disease [7,8]. An inverse association of the measures of body fat distribution and total fat mass with serum iron levels in Hispanic women was established [9].

Various high fat diets with different sources of fat (saturated, and unsaturated) and different proportions of fat of the total energy expenditure are widely used in animals studies to induce obesity [10-13], which is also widely used to examine obesity-induced chronic state of low-grade inflammation, mediated through quantitative and phenotype switching in white adipose tissue macrophages (ATM) [14]. Therefore, the objective of this study was to examine iron homeostasis and tissue iron quantification in female rats after feeding different High-Fat-Diets (HFDs) for six and ten weeks.

MATERIALS AND METHODS

Animals

Eight weeks Sprague-Dawley female rats (36 rats, average weight 105-154 g) were obtained from Jordan University of Science and Technology (JUST), Jordan. Rats were kept at the Animal Unit, School of Agriculture, the University of Jordan, Jordan. Each rat was housed in a single metabolically-ventilated plastic cage (North Kent Plastic Cages, Ltd, Dartford, UK) with a stainless steel wire mesh floor and front. A tray was placed under each cage to collect feces and food spillage. Diet was provided in glass cups and water in glass drinking bottles. Rats were kept under controlled temperature (22 ± 2°C) and maintained at 12:12 hours light: dark cycles with free access to water and standard laboratory chow diet for one week of acclimatization [15].

Experimental design

Rats were randomly divided into three main dietary groups: group (1) rats were fed the high saturated fat diet (HSFD) [n = 12], group (2) rats were fed the High Monounsaturated Fat Diet (HMUSFD) [n = 12] and group (3) rats were fed Normal Fat Diet (NFD) [n = 12]. All rats were fed *ad libitum* for 10 weeks. Animals were weighed weekly during the duration of the experiment. Consumption of diets was measured daily by comparing differences in weight between the amount of food offered and left. After 6 weeks of feeding different diets, 6 rats were selected randomly, sacrificed and samples were collected. The remaining rats were kept on their respective diet completed for 10 weeks. After which, rats were sacrificed by chloroform anesthesia, after 12 hours fasting [15]. Blood samples were collected by cardiac puncture and centrifuged immediately. Serum samples were as kept at -20°C until used [15]. Liver and retroperitoneal adipose tissues (RPAT) were removed [16], rinsed with phosphate buffered saline (PBS) (pH 7.0-7.2), weighed and were kept at -20°C for iron quantification [8].

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Jordan (Permit Number: 27-2956). All surgery was

performed under chloroform anesthesia, and all efforts were made to minimize suffering.

Preparation of diets and diets formulation

A standard American Institute of Nutrition (AIN) diet was used [17]. The modified formulation of AIN-93M diet that replaces casein with egg white solids (AIN-93M-EGG) as a protein source was used [17]. Furthermore, higher amount of tert-butylhydroquinone (TBHQ) was added to high fat diet (HFD) due to their high fat contents and their increased susceptibility to oxidation (2 mg for each 10 g fat) [18]. The estimated minimal nutrient composition for Normal Fat Diet (NFD) group was 14.7% protein (egg white), 75.8% carbohydrates (cornstarch and sucrose) and 9.45% fat (soybean oil); for high monounsaturated fat diet (HMUFD) was 14.7% protein (egg white), 45% fat (35.6% olive oil with 9.4% of fat soybean oil), 40.5% carbohydrates (sucrose and cornstarch) and for High Saturated Fat Diet (HSFD) was 14.7% protein (egg white), 45% fat (35.6% butter milk fat, non-hydrogenated) with 9.4% of fat soybean oil), 40.5% carbohydrates (sucrose and cornstarch) [10]. Total calories for all diets were 3.81 kcal/ g.

BIOCHEMICAL ANALYSIS

Serum iron parameters and tissue iron content

Serum ferritin was examined by using commercially available ELISA kit according to manufacturer's instructions (ab157732 -Ferritin (FTL) Rat ELISA Kit, Abcam, Cambridge, MA, UK). Serum iron and TIBC were determined by quantitative calorimetric analysis using the commercial kit (Interchim kit (No. FT715, av. JF Kennedy, Montlucon, France). Unsaturated iron binding capacity (UIBC) was calculated as follow: UIBC= 500 (the total iron added-excess iron), while TIBC (µg/ dL) = Serum iron (µg/ dL) + UIBC (µg/ dL). For total iron quantification in liver and RPAT, colorimetric iron assay kit (ab83366, Abcam, Cambridge, MA, UK) was used, the absorbance was read at 593 nm and the concentrations were calculated in ng/ml tissue according to the manufacture procedures.

Statistical analysis

Statistical analysis was performed using (SPSS for Windows, Rel. 22.0, 2013, and Chicago: SPSS Inc). Mean differences were examined using one-way Analysis Of Variance (ANOVA) followed by Duncan's post-hoc test for mean separation. Data presented as mean ± SEM and differences between means were considered significant at p-value <0.05.

RESULTS

Table 1 shows no significant changes in all tested iron parameters between all groups during the first six weeks of the treatment ($p > 0.05$). Similarly, Liver Iron Content (LIC) and retroperitoneal adipose tissue iron content RPATIC were not significantly different between all groups of the treatment ($p > 0.05$) as shown in table 3.

Table 2 shows a significant increase in serum ferritin of rats that were fed HFDs as compared to NFD fed group after 10 weeks of the treatment ($p > 0.05$). Moreover, LIC in rats that were fed NFD and HSFD increased significantly as compared to rats that were fed HMUFD ($p > 0.05$) (Table 3).

DISCUSSION

Accumulating evidence suggested that HFDs could affect iron homeostasis negatively, leading to tissue iron accumulation and serum iron deficiency [19,20]; hence, the main objective of this study was to investigate serum iron parameters and tissue iron accumulation after feeding rats with different HFDs for 6 and 10 weeks.

Unlike the findings of Bowering and colleagues [19] who indicated that animals that were fed HFDs for different periods of time has been proved to be affect iron homeostasis, the findings of the current study demonstrated that no significant effect of feeding the HFDs on iron homeostasis or tissue iron deposition after 6 weeks, but the ferritin and LIC were affected significantly after 10 weeks of the treatment. However, the relationship between HFD and hepatic iron remains controversial. While Bowering, et al. [19] reported that hepatic iron concentration was increased in rats fed high fat diet rich in lard for two weeks, Boesch-Saadatmandi and colleagues [21] found that hepatic iron concentration was increased after consumption of the high fat diet rich in tallow for 4 weeks when zinc concentration was low; whereas the findings of the current study showed no significant effect in rats that were fed different types of HFDs and NFD on hepatic iron content after 6 weeks. In addition,

Table 1: The effect of feeding the high-fat-diets for 6 weeks on serum iron parameters.

Variables[A]	Ferritin (ng/ ml)	SI (µmol/ L)	TIBC (µmol/ ml)	UIBC (µg/ dl)
NFD (n = 6)[B]	47.90 ± 2.33[a]	41.33 ± 3.96[a]	50.50 ± 1.55[a]	101.26 ± 20.61[a]
HSFD (n = 6)	53.57 ± 2.86[a]	41.70 ± 2.63[a]	51.75 ± 2.19[a]	85.60 ± 18.63[a]
HUFD (n = 6)	51.25 ± 4.74[a]	33.10 ± 4.71[a]	58.92 ± 2.19[a]	105.20 ± 25.87[a]

A: Data are presented as mean ± SEM, and are significant at $p < 0.05$. Means within the same column with different superscript letters are significantly different.
B: NFD: the normal fat diet; HSFD: the high saturated fat diet; HMUFD: the high monounsaturated fat diet; SI: serum iron; TIBC: total iron binding capacity; UIBC: unsaturated iron binding capacity.

Table 2: The effect of feeding the high-fat-diets for 10 weeks on serum iron parameters.

Variables[A]	Ferritin (ng/ ml)	SI (µmol/ L)	TIBC (µmol/ ml)	UIBC (µg/ dl)
NFD (n = 6)[B]	51.47 ± 3.01[a]	46.85 ± 2.41[a]	59.88 ± 2.74[a]	129.84 ± 21.6[a]
HSFD (n = 6)	77.60 ± 4.14[b]	46.85 ± 2.05[a]	56.38 ± 1.74[a]	133.68 ± 9.08[a]
HUFD (n = 6)	68.81 ± 1.6[b]	42.86 ± 5.51[a]	58.52 ± 1.72[a]	103.06 ± 31.39[a]

A: Data are presented as mean ± SEM, and are significant at $p < 0.05$. Means within the same column with different superscript letters are significantly different.
B: NFD: The Normal Fat Diet; HSFD: The High Saturated Fat Diet; HMUFD: The High Monounsaturated Fat Diet; SI: Serum Iron; TIBC: Total Iron Binding Capacity; UIBC: Unsaturated Iron Binding Capacity.

Table 3: The effect of feeding the high-fat-diets for 6 and 10 weeks on tissue iron parameters.

Period of Treatment	6 weeks		10 weeks	
Variables[A]	LIC (ng/ mg)	RPATIC (ng/ mg)	LIC (ng/ mg)	RPATIC (ng/ mg)
NFD (n = 6)[B]	182.95 ± 8.39[a]	9.99 ± 1.00[a]	168.67 ± 5.42[b]	7.56 ± 0.94[a]
HSFD (n = 6)	165.05 ± 15.05[a]	7.24 ± 0.72[a]	178.16 ± 7.78[b]	6.61 ± 0.89[a]
HUFD (n = 6)	185.01 ± 10.25[a]	9.33 ± 1.04[a]	123.78 ± 6.57[a]	8.47 ± 0.92[a]

A: Data are presented as mean ± SEM, and are significant at $p < 0.05$. Means within the same column with different superscript letters are significantly different.
B: NFD: The Normal Fat Diet; HSFD: The High Saturated Fat Diet; HMUFD: The High Monounsaturated Fat Diet; LIC: Liver Iron Content; RPATIC: Retroperitoneal Adipose Tissue Iron Content

HFDs lead to a systemic iron deficiency in mice by increasing mRNA expression of duodenal iron transporters, and reducing duodenal iron absorption without affecting liver and adipose tissue or serum hepcidin concentrations [20]. Sonnweber, et al. [20] suggested that HFD resulted in iron deficiency due to the consequence of diminished intestinal iron uptake independent of hepcidin and not due to the intake of energy-dense nutrient food or due to higher sequestration in the endothelial cells.

In the current study, the first 6 weeks were not enough to determine the long term effect of the HFD on tissue iron accumulation and iron deposition. However, 10 weeks of feeding different diets resulted in a clear effect on serum ferritin and liver iron deposition. To demonstrate the long term effect of the HFD on iron distribution in the body, Yamano, et al. [22] demonstrated that mice fed the HFD for 18-20 weeks showed increased levels of hemoglobin and serum ferritin, accompanied by iron accumulation in the spleen, without affecting iron content in the heart and liver. Additionally, the long term effects of HFDs on rats were discussed by Dongiovanni and colleagues [23] for 12 weeks; the results showed that hepatic iron accumulation was increased significantly in rats that were fed HFD as compared to rats that were fed regular diet. Moreover, liver iron deposition was paralleled to ferritin levels, consistently with up-regulation of hepcidin, the major iron uptake protein Transferrin Receptor-1 (TfR-1), and the intracellular iron sensor iron regulated protein-1 (IRP1) [23]. Furthermore, Meli and colleagues [24] evidenced an increased time-dependent activity of Iron Regulatory Protein 1 (IRP1) in a liver of HFD fed animals for 8 weeks, associated with the increase in Transferrin Receptor-1 (TfR1) expression and down regulation of Ferritin and Ferroportin (FPN-1) with hepcidin increase [24]. According to our study, liver iron content in rats that were fed HMUFD [rich in olive oil] was not affected as compared to NFD and HSFD after 10 weeks, this sees valid to suggest that type of introducing fat could play a significant impact on tissue iron accumulation, which cannot be ignored and needs more investigation.

The relationship between HFDs, hepatic and serum iron concentrations, and the period of time affecting iron homeostasis remains controversial [20-21] and requires further investigations in this area. Based on the findings of the current study, it sees valid to suggest that introducing saturated or unsaturated HFDs for 6 weeks was not enough to underlie the long term effect of the HFD on tissue iron accumulation and iron deposition.

In conclusion, although HFDs did not affect iron homeostasis and tissue iron deposition in female rats after consumption for short period of time, also, the effect of high-fat-diets on iron homeostasis is time dependent.

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Author's contributions

Buthaina Alkhatib: Designing the research study, conducting the experiment, analyzing data and writing the manuscript.

Hayder Al-Domi: Designing the research study, conducting experiment and language revision.

Basha'er Abu Irmaileh: Data analysis and preparing reagents.

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