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Iron Overload Effect on Serum Levels of Glucose, Insulin and HOMA-IR was Influenced by the Type of Fat in Female Sprague-Dawley Rats Fed High-Fat-Diets

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Abstract

Objective: This study was conducted to examine the effect of iron supplementation on serum levels of glucose, insulin and HOMA-IR, and to examine the histological iron deposition in tissue in female Sprague-Dawley rats fed high-fat-diets (HFDs).

Methods: Rats were divided into three main groups: normal fat diet (NFD), high saturated fat diet (HSFD) and high monounsaturated fat diet (HMUSFD). After 6 weeks, rats were sub-divided into three sub-groups for intraperitoneal injection of iron (control group, dose1 group (Do1); 15 mg/kg body weight (BW) and dose 2(Do2); (75 mg/kg BW). Serum glucose and insulin were measured. Also, the histological examination of iron deposition after Perl's Prussian staining was examined.

Results: We indicated that a high dose of iron supplementation decreased total weight gain significantly by 3 folds in rats that were fed the NFD and 2.5 folds in rats that were fed the HMUSFD as compared to control sub-groups ($p < 0.05$). Moreover, mean HOMA-IR levels increased significantly in rats received a high dose of iron as compared to controls in the NFD and the HSFD groups ($p < 0.05$) but not in HUSFD. However, liver iron accumulation was increased in HFDs groups compared to NFD.

Conclusion: The findings showed that iron overload complications could be influenced by the degree of saturation of dietary fat. However, the high dose of iron supplementation decreased total weight gain and increased liver weight, accompanied by insulin resistance. It is noteworthy that the diet rich in olive oil could have a protective effect against insulin resistance and adipose tissue iron accumulation.

Keywords: Glucose; HOMA-IR; Hfds; Insulin; Tissue iron deposition

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Introduction

Iron deficiency is the most common cause of anemia worldwide [1]. Globally, iron deficiency anemia affect more than two billion people especially the children due to their higher iron requirements [2]. Moreover, iron deficiency anemia is associated with a decrease in the cellular immune response, mental function, physical activity, and alterations in hormonal regulation [3]. Therefore oral iron supplementation is commonly prescribed for people diagnosed with anemia [1]. Findings of recent

reports demonstrated that iron supplementation has complex interactions between diet, the host immune system and the gut micro-biome [4]. The amount of supplemental iron absorbed in the human gastrointestinal tract is low, most of the dose passes into the colon where it becomes available for the pathogenic bacteria [5], which lead to alteration in the composition of the gut microbiota in malnourished children [6].

Oral iron intake could alter gut function and microbial composition through direct induction of reactive oxygen species leading to

increased cell stress in enterocytes and adversely affects the gut micro-biome, increasing pathogen abundance and causing intestinal inflammation [4]. Iron fortification in rural areas resulted in a significant increase of infection-related mortality, mostly related to malaria and invasive bacterial infections, produced potentially pathogenic gut microbiota profile, up-regulation of gut inflammation or increased morbidity due to diarrhea [7]. Dongiovanni and his colleagues [7] found that iron supplementation increased hepatic iron and serum hepcidin fivefold and led to a 40% increase in fasting glucose in mice. However, iron-supplemented mice had lowered visceral adipose tissue mass, associated with iron accumulation in adipocytes. Moreover, iron-enriched diets up-regulated iron responsive genes and adipokines, favoring insulin resistance [8]. Additionally, Gao and his collaborates [8] found that dietary iron supplementation is associated with increased appetite, thus, serum ferritin was negatively associated with serum leptin in a cohort of patients with metabolic syndrome [9]. However, moderately elevated iron levels are associated with chronic diseases such as atherosclerosis, type 2 diabetes mellitus (T2DM) and premature death [10]. Dysmetabolic iron overload syndrome (DIOS) is characterized by increased ferritin levels, and increased body iron stores in the presence of insulin resistance [8]. Moreover, DIOS with normal or mildly elevated transferrin saturation was observed in approximately a one-third of patients with metabolic syndrome or nonalcoholic fatty liver disease [11].

Iron overload can affect major tissues involved in glucose and lipid metabolism, as well as, organs affected by chronic diabetic complications [12]. Epidemiological studies showed an association between iron stores and the development of metabolic syndrome [13]. Zheng and colleagues [13] noted that liver iron is increased in people with T2DM and insulin resistance [14]. Moreover, increased body iron stores were significantly associated with risk of T2DM [15]. Recently, it was reported that the serum concentration of prohepcidin (a precursor of the mature hepcidin) was significantly higher in males with impaired glucose tolerance, or T2DM than in those with normal glucose tolerance [16].

In liver, iron overload can disrupt insulin inhibition of hepatic glucose production, which together with reduced hepatic extraction of insulin leads to peripheral hyperinsulinemia [17]. Moreover, an iron-enriched diet leads to iron accumulation and insulin resistance in visceral AT in mice [8].

Adipose tissue also seems to have an active role in the modulation of systemic iron metabolism through the production of adipokines, which, interacts with iron metabolism; which suggests that iron overload could contribute to obesity associated AT dysfunction [18]. As such, iron overload could disrupt insulin activity in muscle possibly by activation of stress pathways with generation of ROS, which, lead to the hydroxylation of phenylalanine residues of insulin, and therefore, promotes insulin resistance [19]. Therefore, the objective of this study was to examine the effect of different doses of iron supplementation on serum levels of glucose, insulin and HOMA-IR, and to examine the histological iron deposition in tissue in female Sprague-Dawley rats fed high-fat-diets.

Materials and Methods

Preparation of diets and diets formulation

A standard American Institute of Nutrition (AIN) diet was used [20]. The modified formulation of AIN-93M diet that replaces casein with egg white solids (AIN-93M-EGG) as a protein source was used [20] as shown in (Table 1). 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) [21]. 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) [22] 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) [23]. Total calories for all diets were 3.81 kcal/g.

Animals: Eight weeks Sprague-Dawley female rats (54 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 ($22 \pm 2^\circ\text{C}$) and maintained at a 12:12 hours light: dark cycles with free access to water and standard laboratory chow diet for one week of acclimatization [24]. 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).

Iron supplementation: Iron sucrose injection (Vanofer/Vifer International Inc., St. Gallen, Switzerland) was used. Each ml of Vanofer contains 20 mg elemental iron as iron sucrose in water for injection. Each dose of iron was calculated and prepared up to possible volume depended on rat weight before each injection using insulin syringe (1 mL) [25].

Experimental design: Rats were randomly divided into three main dietary groups: group (1) rats were fed normal fat diet (NFD) (n=18), group (2) rats were fed the high saturated fat diet (HSFD) (n=18) and group (3) rats were fed the high monounsaturated fat diet (HMUSFD) (n=18). All rats were fed *ad libitum* for 10 weeks. Animals were weighed weekly during the duration of the experiment and three times per week before each injection of iron dose in the last 4 weeks 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, rats were sub-divided into three sub-groups (control group, dose1 group and dose2 group; n=6) for the second stage of study (4 weeks) with two doses of iron

supplementation. Rats were kept on their respective diet. Iron was supplemented by intraperitoneal injections as two doses of iron sucrose: dose1 (Do1); 15 mg/kg body weight (BW) and dose 2(Do2); (75 mg/kg BW), three times per week, for 4 consecutive weeks [25-27]. In control group, rats received an equivalent volume of sterile saline

After which, rats were sacrificed by chloroform anesthesia, after 12 hours fasting. Blood samples were collected by cardiac puncture and centrifuged immediately. Serum samples were as kept at -20°C until used [24].

Biochemical analysis

Glucose analysis: Glucose serum levels were examined by glucose enzymatic reference method with hexokinase using commercial kit (Teco Diagnostics, Lakeview Ave, Anaheim, USA). The absorbance was read against the blank (1000 µl reagent) at 340 nm with a spectrophotometer (Spectroscan 80D, Biotech Engineering Management Co. Ltd, UK) then values were calculated in mmol/L.

Serum insulin and HOMA-IR: Serum insulin was measured by commercially available ELISA kit (ELISA Abcam's Rat Insulin Kit, ab100578, Abcam, Cambridge, MA, UK) then the concentrations were calculated in µIU/ml. Insulin resistance was evaluated by the homeostasis model assessment of insulin resistance method (HOMA-IR) described by Matthews et al. (1985). The HOMA-IR index was calculated as follows: (fasting insulin (µIU/ml) x fasting glucose (nmol/ml))/22.5 [28].

Histological examination: Liver and RPAT samples were fixed in 10% PBS (pH 7.0-7.2) buffered formalin. All tissues were embedded in paraffin after fixation. Histologic staining was performed using Eosin and Perl's Prussian blue method to detect tissue iron deposition [25,8]. Tissue samples were examined under light microscope at 200x and 400x (OLYMPUS, USA).

Statistical Analysis

Statistical analysis was performed using (SPSS for Windows, Rel. 22.0, 2013, Chicago:SPSS Inc). Mean differences were examined using one-way analysis of variance (ANOVA) followed by Tukey's HSD test for mean separation. Shapiro-Wilk test was used to assess normality of distribution. Data are presented as mean ± SEM and differences between means were considered significant at *p*-value<0.05.

Results

Table 2 shows that mean TRPAT weight and total weight gain in NFD-Co sub-group were significantly higher compared to NFD-Do1 and NFD-Do2 sub-groups (*p*<0.05). However, mean final weight and liver weight of NFD-Co sub-group were significantly higher than NFD-Do2 sub-group, but not NFD-Do1 sub-group (*p*<0.05).

There were significant differences in mean TRPAT weight and total weight gain between the HSFd sub-groups; which were significantly higher in HSFd-Co sub-group as compared to

Table 1 The composition of the normal fat diet (NFD) and the high-fat diets (HFDs).

Type of diet	NFD (AIN-93M)		High-Fat Diets			
	Ingredients	Weight (g\kg)	Kcal%	HSFD		HMUFD
Weight (g\kg)				Kcal%	Weight (g\kg)	Kcal%
Cornstarch ¹	612.5	64.4	273.8	28.8	273.8	28.8
Egg white	140	15.0	140	14.7	140	15.0
Sucrose	100	10.5	100	10.5	100	10.5
Soybean oil ²	40	9.5	40	9.5	40	9.5
Olive oil	-	-	-	-	150.5	35.6
Butter ³	-	-	150.5	35.6	-	-
Fiber (α-cellulose)	50	-	50	-	50	-
Mineral mix	35	-	35	-	35	-
Vitamin mix	10	1.05	10	1.05	10	1.05
Biotin premix ⁴	10	-	10	-	10	-
Choline	2.5	-	2.5	-	2.5	-
TBHQ ⁵	8 mg	-	38.2 mg	-	38.2 mg	-
Total weight (g)	1000	-	812.0	-	812.0	-
Total energy (Kcal)	-	3812	-	3812	-	3812

NFD): the normal fat diet, (HSFD): the high saturated fat diet, (HMUFD): the high monounsaturated fat diet.

1. The amount of cornstarch include 155 g extra of cornstarch used as substitution for the dextrinized cornstarch because it is used for facilitating pelleting while no pelleting was done here.
2. Crude refine soybean oil without added vitamins was used in the current study.
3. Butter melted on a water bath.
4. Biotin premix was made by mixing 130 mg of biotin with 999.87 g of cornstarch (Reeves, 1996).
5. The amount of Tert-butylhydroquinone (TBHQ) was added according to the amount of fat (2 mg for each 10 g fat).

Table 2 The effect of the HFDs in combination with iron supplementation on mean liver weight, TRPAT weight, final weight and total weight gain for 4 weeks.

Variables*	Sub-groups (n)	Weights before iron supplementation (g)	Liver Weight (g)	TRPAT wt. (g)	Final Weight (g)	Total Weight Gain (g)
NFD	NFD-Co (8)	203.0 ± 6.80 ^a	5.95 ± 0.2 ^a	3.00 ± 0.36 ^c	219.4 ± 8.3 ^b	12.0 ± 1.6 ^c
	NFD-Do1 (8)	203.0 ± 4.37 ^a	6.48 ± 0.2 ^{ab}	2.28 ± 0.20 ^b	206.3 ± 2.2 ^{ab}	0.6 ± 2.4 ^b
	NFD-Do2 (8)	203.1 ± 3.15 ^a	6.95 ± 0.3 ^b	0.98 ± 0.08 ^a	202.3 ± 4.0 ^a	-6.8 ± 2.2 ^a
HSFD	HSFD-Co (7)	200.0 ± 8.9 ^a	6.58 ± 0.5 ^a	3.84 ± 0.40 ^c	210.6 ± 12.6 ^a	10.2 ± 3.5 ^a
	HSFD-Do1 (7)	201.1 ± 11.3 ^a	7.27 ± 0.5 ^a	2.41 ± 0.23 ^b	214.9 ± 8.2 ^a	9.2 ± 2.3 ^a
	HSFD-Do2 (7)	200.0 ± 8.4 ^a	7.55 ± 0.4 ^b	1.93 ± 0.23 ^a	206.8 ± 8.7 ^a	6.9 ± 6.0 ^b
HMUFD	HMUFD-Co (7)	206.3 ± 8.9 ^a	7.57 ± 0.3 ^b	3.88 ± 0.3 ^b	230.14 ± 6.6 ^a	23.0 ± 5.7 ^b
	HMUFD-Do (8)	207.3 ± 6.3 ^a	8.19 ± 0.4 ^a	2.74 ± 0.2 ^a	225.63 ± 5.0 ^a	19.2 ± 1.9 ^b
	HMUFD-Do2 (7)	207.1 ± 7.8 ^a	8.53 ± 0.3 ^a	2.41 ± 0.3 ^a	219.57 ± 8.4 ^a	9.3 ± 2.7 ^a

*Data are presented as mean ± SEM, and is significant at $p < 0.05$. Means within the same column with different superscript letters are significantly different.

NFD: normal fat diet; HSFD: high saturated fat diet; HMUFD: high monounsaturated fat diet; TRPAT wt.: total retroperitoneal adipose tissue weight; Co: control sub-group without iron supplementation; Do1: iron supplementation dose1 (15 mg/kg); Do2: iron supplementation dose2 (75 mg/kg).

HSFD-Do1 and HSFD-Do2 sub-groups ($p < 0.05$). Moreover, mean TRPAT weight in HSFD-Do1 sub-group was significantly higher than HSFD-Do2 sub-group and mean total weight gain in HSFD-Do2 sub-group was significantly lower than HSFD-Do1 sub-groups ($p < 0.05$). Mean liver weight in HMUFD-Co sub-group was significantly lower than HMUFD-Do1 and HMUFD-Do2 sub-groups, while mean TRPAT weight in HMUFD-Co sub-group was significantly higher than HMUFD-Do1 and HMUFD-Do2 sub-groups ($p < 0.05$). Furthermore, mean total weight gain in HMUFD-Do2 sub-group was significantly lower than HMUFD-Co and HMUFD-Do1 sub-groups ($p < 0.05$).

Table 3 shows that mean FBG in dose-2 sub-groups in the NFD and the HSFD was increased significantly as compared to the control sub-group, while HOMA-IR levels were significantly increased as compared to the control group in the HSFD and compared to dose-1 in the NFD group. However, in rats that were fed the HMUFD, mean HOMA-IR levels were significantly decreased compared to control and dose-1 sub-groups ($p < 0.05$).

Furthermore, iron accumulations were increased in liver at different doses of iron supplementation in the HFDs groups as compared to controls and the NFD groups (**Figure 1**). In contrast, rats that were fed HSFD and NFD accompanied by the higher dose of iron supplementation recorded the highest iron accumulation in RPAT as compared to HMUFD-Do2 (**Figure 2**).

Discussion

Obesity is a state of chronic subclinical inflammation [29], leading to anemia of chronic disease [30]. Evidence indicated that iron overload leads to adipose tissue and endocrine dysfunction; this could affect adipokines secretion and/or interrupt insulin signals pathways leading to obesity-related diseases [31]. It is therefore pivotal to investigate the effect of different types of fat in combination with iron overload, which could provide an opportunity to develop preventative and even therapeutic approaches, to reduce the risk of obesity associated metabolic consequences if present.

The findings of the current study demonstrated that injecting rats with different doses of iron supplementation (15 mg/kg body weight and 75 mg/kg body weight) decreased total weight gain and RPAT weight, accompanied by increasing liver weight regardless of the diet type, compared to controls within group. With reference to the findings of previous researches, this is consistence with the findings of a study conducted on mice fed iron enriched diet, mice gained less weight than controls, accompanied by decreased in visceral adipose tissue mass, and a reduction in the mean size of visceral adipose tissue, by reducing energy intake and body weight gain [32,8].

Moreover, Gabrielsen et al. [8] reported that the effects of iron on adipose tissue mass are mediated by down-regulation of adiponectin release; by which, fat mass was reduced in iron-overloaded mice [31,33]. Hence, adiponectin contributed to increase the rate of fatty acid oxidation through increased adenosine monophosphate activated protein kinase (AMPK) activation and decreased acetyl Co-A carboxylase (ACC) activity [33,34].

Furthermore, Huang et al. [18] showed that mice fed high-fat-high-iron-diet exhibit a shift from glucose to fatty acid oxidation in fuel performance; however, the decrease in glucose oxidation in muscle is accompanied by increase pyruvate/lactate recycling to liver, and increased hepatic glucose output and protect from diet induced obesity [35]. Also, this mechanism has been explained by the modest degree of mitochondrial dysfunction; it relatively contributed to increase the capacity of fatty acid oxidation in mice fed high-fat diets [35,32]. Mice fed high-fat diet with excess dietary iron intake, showed an increase in serum glucose and insulin accompanied by greater hepatic fat accumulation, paralleled to alterations in the hepatic gluconeogenesis enzymes and lipid synthesis due to oxidative stress and mitochondrial dysfunction [32].

The increase of iron dose was accompanied by increase in liver weight in the current study; this was explained by the association

Table 3 The effect of the normal-fat-diet in combination with iron supplementation on mean fasting blood glucose, fasting insulin and HOMA-IR levels.

Variables*	Sub-groups (n)	FBG (mmol/L)	Fasting Insulin (μIU/ml)	HOMA-IR
NFD	NFD-Co (8)	10.09 ± 0.52 ^a	16.09 ± 1.86 ^a	7.49 ± 1.52 ^{ab}
	NFD-Do1(8)	11.54 ± 0.42 ^{ab}	13.48 ± 0.30 ^a	6.95 ± 0.36 ^a
	NFD-Do2 (8)	12.62 ± 0.55 ^b	14.16 ± 0.38 ^a	8.08 ± 0.54 ^b
HSFD	HSFD-Co (7)	10.31 ± 0.29 ^a	13.68 ± 0.35 ^a	6.28 ± 0.22 ^a
	HSFD-Do1 (7)	10.53 ± 0.42 ^a	13.79 ± 0.17 ^a	6.51 ± 0.32 ^a
	HSFD-Do2 (7)	13.99 ± 0.57 ^b	13.24 ± 0.15 ^a	8.19 ± 0.33 ^b
HMUFD	HMUFD-Co (7)	12.83 ± 0.41 ^b	14.02 ± 0.48 ^a	7.99 ± 0.40 ^b
	HMUFD-Do1 (8)	12.59 ± 0.25 ^b	13.45 ± 0.18 ^a	7.52 ± 0.17 ^b
	HMUFD-Do2 (7)	11.24 ± 0.39 ^a	13.65 ± 0.15 ^a	6.63 ± 0.29 ^a

*Data are presented as mean ± SEM, and is significant at $p < 0.05$. Means within the same column with different superscript letters are significantly different.

NFD: normal fat diet; HSFD: high saturated fat diet; HMUFD: high monounsaturated fat diet; Co: control sub-group without iron supplementation; Do1: iron supplementation dose1 (15 mg/kg); Do2: iron supplementation dose2 (75 mg/kg); FBG: fasting blood glucose; HOMA-IR: homeostatic model assessment for insulin-resistance.

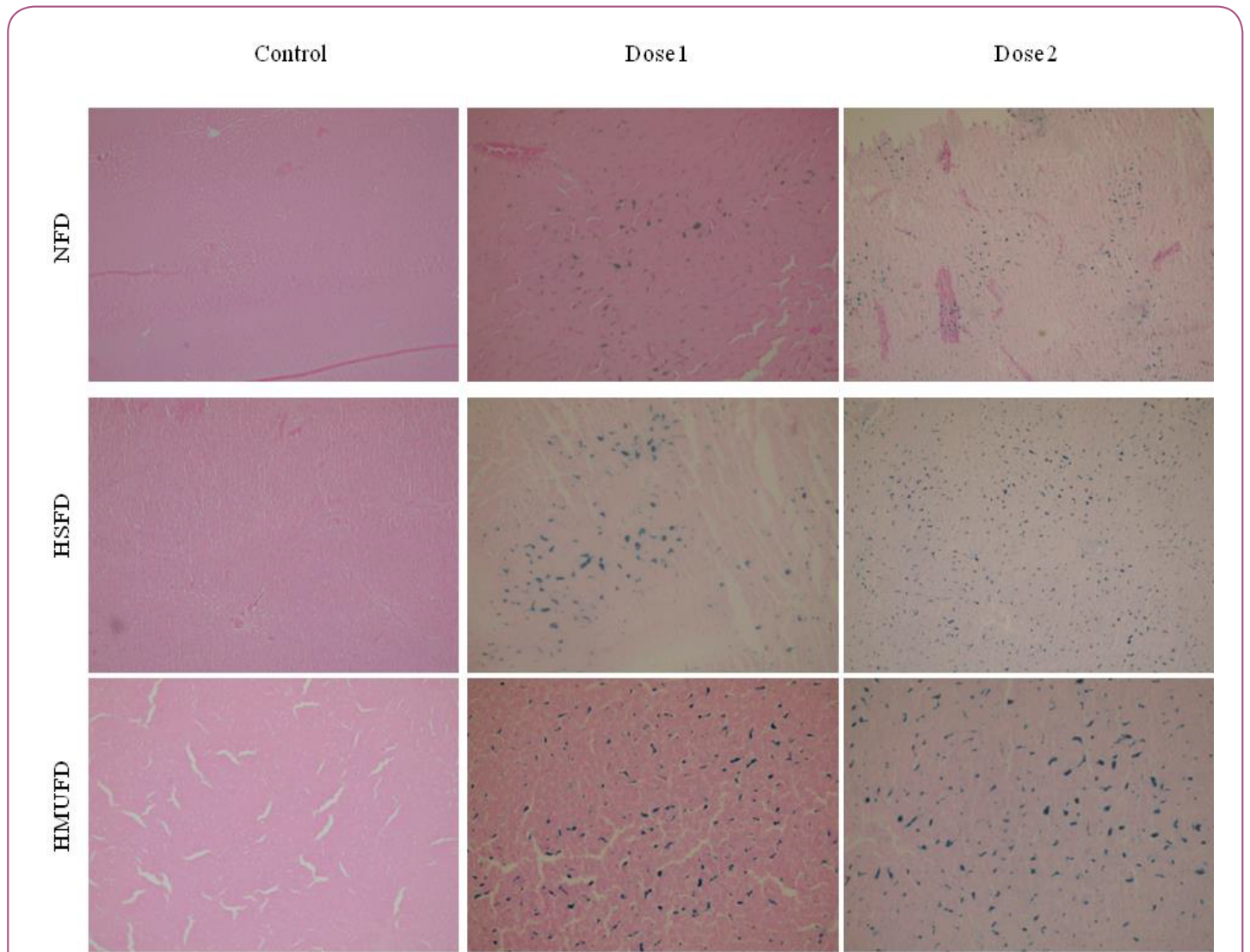


Figure 1 Iron deposition in liver after feeding the HFDs in combination with two doses of iron supplementation for 4 weeks (x200 and x400). Control sub-groups without iron supplementation; iron supplementation-Do1 sub-groups (15 mg/kg); iron supplementation-Do2 sub-groups (75 mg/kg); NFD: normal fat diet; HSFD: high saturated fat diet; HMUFD: high monounsaturated fat diet; sections were subjected to Perl's Prussian blue staining.

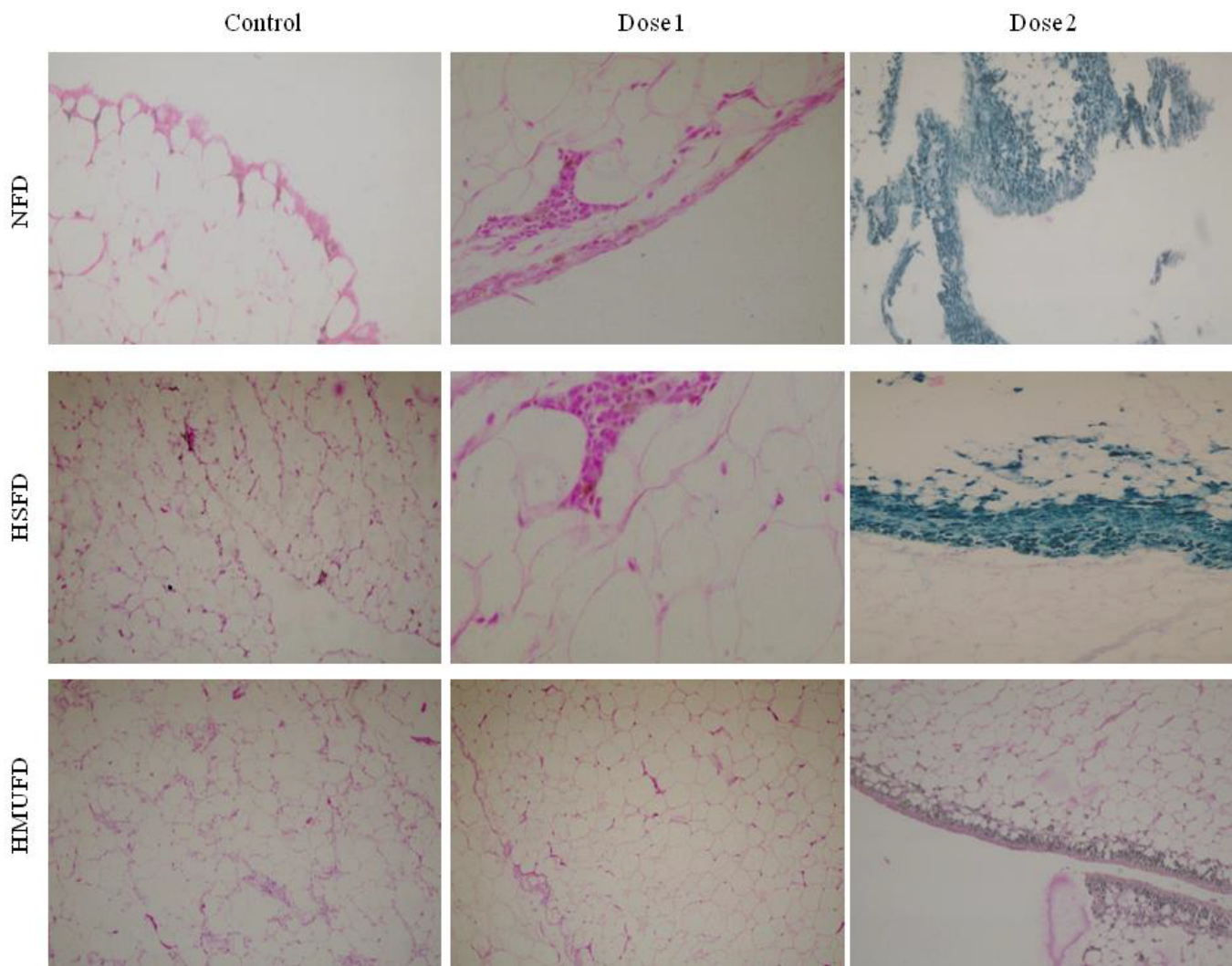


Figure 2 Iron deposition in RPAT after feeding the HFDs in combination with two doses of iron supplementation for 4 weeks (x200 and x400). Control sub-groups without iron supplementation; iron supplementation-Do1 sub-groups (15 mg/kg); iron supplementation-Do2 sub-groups (75 mg/kg); NFD: normal fat diet; HSFD: high saturated fat diet; HMUFD: high monounsaturated fat diet; sections were subjected to Perl's Prussian blue staining.

between iron overload and liver fibrosis, which increased liver weight of non-alcoholic fatty liver disease and non-alcoholic steato-hepatitis [12,36]. Furthermore, hepatic fat accumulation accompanied by the iron supplementation could increase liver weight [37], however, in a mice fed the HFD and low-fat diet with dietary iron supplementation, greater hepatic fat was accumulated in all supplemented groups compared to control groups, with respect to the type of diet. These changes were paralleled by alterations in the levels of hepatic gluconeogenesis enzymes, and lipid synthesis [32]. It has been showed that the increase in liver size results from hepatic cellular hypertrophy accompanied by lipid deposition in the liver lobule [38].

The findings of the present study demonstrated that the accumulation of iron in liver after iron overloaded was increased in rat fed HFDs compared to NFD (**Figure 1**). This was shown in a study on rats by which rats that were fed HFD accumulated more hepatic iron than those fed regular diet, which was associated with development of steatosis [39].

The current study showed that high dose of iron supplementation in the NFD and HSFD decreased insulin sensitivity paralleled to increase iron deposition in RPAT. This result was expected according to what has been reached in many earlier studies to clarify the relationship between the high iron intake or iron overload and insulin insensitivity [8,31,32,40,41]. The study of Mendler's et al. [41] demonstrated that patients with unexplained hepatic iron overload were characterized by a mild to moderate iron burden, and that was associated to insulin resistance irrespective of liver damage. In order to explain the association between body iron store and the risk of T2DM [42], Green et al. [39] concluded that transferrin and iron were induced insulin resistance in adipocytes through a mechanism independent of fatty acids [40]. Moreover, Green et al., [39] and Jiang et al. [42] showed that higher iron stores in liver and adipose tissue were associated with increased risk of T2DM in healthy women [40,43].

On the other hand, an epidemiological study showed an association between iron stores and the development of

metabolic syndrome [13]. Iron overload in liver can disrupt insulin inhibition of hepatic glucose production, which together in conjunction with reduced hepatic extraction of insulin, leads to peripheral hyper-insulinemia [17]. Zheng and his colleagues [13] noticed that liver iron content was increased in people with T2DM and insulin resistance [14], similarly, Bao and colleagues concluded that increased body iron stores were significantly associated with the risk of T2DM [15].

Recently, a study was conducted on mice, by which mice fed the HFD for 7 weeks with dietary iron supplementation, showed that the high iron levels increased blood glucose but decreased high-density lipoprotein cholesterol levels [32]. In mice fed the high iron diet, iron negatively regulated adiponectin transcription and increased insulin resistance, and they demonstrate a causal role for iron as a risk factor for metabolic syndrome [31]. Similarly, iron-enriched diet induces insulin resistance and hypertriglyceridemia [8].

Furthermore, Fargion et al. [35] demonstrated that iron status affects insulin sensitivity by modulating the transcription and membrane affinity of insulin receptor expression in the liver, and influencing insulin-dependent gene expression [36], but Manco et al. [43] was not able to observe a significant difference between insulin sensitivity in patients with and without hepatic siderosis at short period of time, after assessing the intra-hepatic iron in non-alcoholic fatty liver disease (NAFLD) patients [44].

Our results are in line with previous reports in literature which demonstrated the high dose of iron supplementation with the NFD and HSFD groups decreased insulin sensitivity and increased iron accumulation in adipose tissue and major tissue involved in glucose and lipid metabolism [8,31,44] but this was not the case in the HMUFD group by which the diet was rich in olive oil (35.6%).

Surprisingly, the HMUFD in combination with high dose of iron supplementation protected rats against insulin insensitivity and prevented severe iron accumulation in adipose tissue. This demonstrated that the type of fat that was introduced made an impact that cannot be ignored. It could have a significant effect on the response to the high iron diet or iron supplementation, and consequently affect insulin sensitivity. By reference to the articles that were discussed the role of fat type in insulin resistance, Storlien et al. [44] found that not all types of fat lead to insulin resistance in rats [45]. In addition, Miret et al. [45] demonstrated that olive oil-rich diet did not increase oxidative stress compared to fish oil-rich diet. It has been found that olive oil-rich diet

decreases accumulation of triglycerides in liver, accompanied by improving postprandial triglycerides, glucose and glucagon like peptide-1 responses in insulin-resistant subjects [46].

However, most of the conducted studies in human and animals confirmed the olive oil role in protecting against NAFLD, decreasing blood triglycerides, increasing high-density lipoprotein, reducing insulin resistance and hepatic steatosis [47-51]. It has showed that consumption of diets that rich in olive oil has a profound effect on different health outcomes, specially obesity, T2DM and metabolic syndrome [52]. In this line, hydrophilic phenols are the most common natural antioxidants of olive oil, which give it many important biological features, especially antioxidant effect, anti-inflammatory, chemo-preventive and anti-cancer [52].

Up to our knowledge, this is the first study that demonstrated the protective role of olive oil in preventing insulin resistance induced by iron overload. Further clinical trials to examine the association between types of dietary fat consumed and deposition in different tissues, and dietary fat assessment on individuals are warranted.

Conclusions

A high dose of iron supplementation accompanied with HFDs lead to decrease total weight gain and adipose tissue weight significantly, whereas liver weight increased regardless of the diet type. The type of dietary fat that was introduced could have a crucial effect on the response to the high iron diet or iron supplementation, which consequently could enhance insulin sensitivity, tissue iron accumulation and anti-obesity effect.

Author's Contributions

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

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

Maha Shomaf: microscope techniques, preparation of tissue slides and staining, reading the slide of histopathology

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

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