

Chapter one

Introduction and literature review

1- Introduction and literature review

1-1 Introduction

Iron is an important dietary mineral that is involved in various body function, including the transport of oxygen in the blood. This is essential in providing energy for daily life. Keeping the iron levels within healthy limits is important. Most of the body iron stores are within the hemoglobin of red blood cells, and carry oxygen to the body. Extra iron is stored within the liver and is used during times when dietary intake is inadequate. (Malcolm; *et al.*2015).

Both iron deficiency (ID) and obesity are global epidemics affecting billions with regional disparities (Low, *etal.* 2009).It has become clear that iron deficiency and obesity do not merely represent the coincidence of two frequent conditions but are molecularly linked and mutually affect each other (Felder; *et al.*2013). While obesity has become a socioeconomic burden in industrialized countries over the last century, the prevalence Is currently also increasing in developing countries with the spread of energy-dense food compounds and assed entary life style (Lear;*et al.*2014).The interaction of iron homeostasis with obesity represents a Janus-faced clinical condition. On the one hand, obesity may promote iron deficiency by inhibition of dietary iron uptake from the duodenum. On the other hand, a condition termed “dysmetabolic iron overload syndrome (DIOS)” has become the most frequent differential diagnosis for elevated ferritin concentrations, affecting approximately one-third of subjects with nonalcoholic fatty liver disease (NAFLD) or metabolic syndrome (MetS). DIOS is characterized byincreased serum ferritin concentrations with normal or mildly elevated transferrin saturation in subjects with various components of MetS or NAFLD. True iron overload is rarely found in liver biopsy, and liver iron only

weakly correlates with serum ferritin concentrations. It is associated with more severe manifestations and outcomes. From a therapeutic perspective, iron removal via phlebotomy has been established to convey additional benefit (Felder.*et al.*2013). From a clinical point of view, both conditions are relevant and require adequate diagnostic work-up and treatment. This review is mainly focused on iron deficiency (ID) in obesity and aspects of DIOS are only mentioned where relevant to the main topic. The obesity is defined by body mass index (BMI) or Quetelet index which is value derived from the mass (weight) and height of an individual. The BMI is define as the body mass individual by the square of the body height , and is universally expressed in units of kg/m^2 , resulting from mass in kilograms and height in meter .The BMI an attempt to quantify the amount of tissue mass (muscle ,fat and bone) in an individual , and then categorize that person a underweight ,normal weight, overweight or obese base on that value. (Malcolm;*et al.*2015)However, there is some debate about where on the BMI scale the dividing line between categories should be placed. The Commonly accepted BMI ranges are underweight: under 18.5, normal weight: 18.5- 25, overweight: 25 - 30, obese 30-40, over obese >40. (Malcolm; *et a.l*2015).

1-2 Literature review

1-2-1 Iron Role in the Body:

Iron is an essential element for most life on Earth, including human beings.

Iron is needed for a number of highly complex processes that continuously take place on a molecular level and that are indispensable to human life, e.g. the transportation of oxygen around your body.

Iron is required for the production of red blood cells (a process known as hematopoiesis), but it's also part of hemoglobin (that is the pigment of the red blood cells) binding to the oxygen and thus facilitating its transport from the lungs via the arteries to all cells throughout the body.

Once the oxygen is delivered the iron (as part of haemoglobin) binds the carbon dioxide which is then transported back to the lung from where it gets exhaled.

Iron is also involved in the conversion of blood sugar to energy. Metabolic energy is crucial for athletes since it allows muscles to work at their optimum during exercise or when competing.

The production of enzymes (which play a vital role in the production of new cells, amino acids, hormones and neurotransmitters) also depends on iron, this aspect becomes crucial during the recovery process from illnesses or following strenuous exercise or competing.

The immune system is dependent on iron for its efficient functioning and physical and mental growth require sufficient iron levels, particularly important in

childhood and pregnancy, where the developing baby solely depends on its mother's iron supplies.

Iron is lost by the body through a variety of ways including urination, defecation, sweating, and exfoliating of old skin cells. Bleeding contributes to further loss of iron which is why women have a higher demand for iron than men.

If iron stores are low, normal hemoglobin production slows down, which means the transport of oxygen is diminished, resulting in symptoms such as fatigue, dizziness, lowered immunity or reduced ability for athletes to keep up with their training programs.

1-2-2 Physiology of Iron Metabolism

Many aspects of the physiological regulation of human iron homeostasis have been elucidated over the past decade.(Hentze;*et al.*2010). Iron is absorbed as Fe^{2+} in the proximal duodenum by the divalent metal transporter 1(DMT1) (Gunshin,*et al.*1997). Following its transfer through the duodenal basolateral membrane facilitated by the iron exporter ferroportin(FPN) (Abboud and Haile .2000). Iron undergoes oxidation by the membrane-bound copper containing ferroxidas hephaestin.(Vulpe;*et al.*1999). Before being incorporated into transferrin for further transport into circulation.

Although heme constitutes an important source of iron from the diet and also supplies iron for cellular iron requirements via re-utilization, the mechanism for enteral heme uptake has not yet been identified. .(Korolnek and Hamza;2014). Most cells acquire iron via the uptake of transferrin-bound iron (Fe^{3+}) by the transferrin receptor (TfR1). Iron is mainly required for hemebiosynthesis in the erythropoietic bone marrow and other heme containing enzymes (e.g.,

cytochromes), whereas excess iron is stored in the liver hepatocytes. Iron is exported from hepatocytes, macrophages and all other mammalian cells via FPN, which has thus far been identified as the only iron exporter..(Hentze;*et al.*2010).

Systemic iron homeostasis is maintained in a hormone-like negative feedback mechanism by the 25-amino acid peptide hormone hepcidin (hepatic bactericidal protein) (Ganz and Hentze, 2003). Hepcidin is secreted from hepatocytes in response to iron overload, inflammation, hypoxia or anemia. Hepcidin exerts its regulatory functions on iron homeostasis via binding to FPN, thereby leading to FPN phosphorylation, degradation and consequently to blockage of cellular iron export which induces a decrease in serum iron (Nemeth, *et al*, 2004).

Although in quantitative terms the liver is the main source of circulating hepcidin, Macrophages, pancreatic islet cells and adipose tissue can also express hepcidin (Bekri, *et al*, 2006).

1-2-3 Iron Phenotype of Obesity

It appears counterintuitive that obesity as a condition of calorie and nutrient excess is associated with ID. However, the profound changes in energy homeostasis in AT, the liver and also other organs involved are closely linked to distinct changes of iron homeostasis. Iron dysregulation in obesity may, in the manner of the Roman god Janus, present with two sides facing opposite directions. Data from pathophysiological studies strongly suggest that these two distinct clinical problems are in fact manifestations of the same underlying mechanisms with obesity-related iron deficiency on one side and DIOS on the other. It appears reasonable to assume that the observed iron phenotype finally results from the net balance of often competing stimuli.

1-2-4 Mechanisms of underlying iron deficiency in obesity

The central finding of studies examining iron homeostasis in obese subjects represents an impaired ability of duodenal iron absorption. Markedly lower isotope-labeled iron absorption in obese compared to overweight and normal weight subjects with or without ascorbic acid was found (Mujica, *et al*, 2014). Similar observations were reported in obese men and children from subsequent investigations. (Ruz, *et al*, 2012). Thus, decreased dietary iron uptake due to lower enterocyte iron absorption can be regarded as the pathophysiological hallmark of iron dysregulation in obesity. Since the liver-derived peptide hormone hepcidin represents the master regulator of iron homeostasis, its role has been investigated in the context of obesity. Elevated serum hepcidin concentrations have been reported in severe obesity with associated anemia (Bekri, *et al*, 2006), but also in studies investigating mechanisms underlying the DIOS. (Aeberli, *et al*, 2009). As decreased enteral iron absorption and elevated hepcidin expression are found in both obesity-related iron deficiency and DIOS, we suggest that these conditions represent different manifestations of the same underlying pathophysiological process. (Mujica, *et al*, 2014). While elevated hepcidin in DIOS is related to increasing iron stores, in severe obesity with iron deficiency it appears primarily linked to inflammatory markers. (McClung and Karl, 2009). Irrespective of the underlying cause, elevated hepcidin concentrations explain lower duodenal FPN expression and diminished dietary iron absorption. Although the liver is traditionally regarded as the main source of hepcidin, expression of hepcidin in the adipose tissue from anemic, iron-poor, morbidly obese subjects has been demonstrated which is absent in lean adipose tissue (Bekri, *et al*, 2006). As AT mass increases, the contribution of AT-derived hepcidin may become substantial, although the relevance of AT hepcidin has been questioned by a

recent investigation due to very low amount of hepcidin mRNA compared to expression in the liver and lack of evidence of a substantial release from AT. (Tussing, *et al*, 2011). It appears plausible, however, that AT-derived cytokines such as IL-6 and IL-1 function as potent inducers of hepcidin expression in the liver also in obesity (Ganz and Nemeth, 2009). This conclusion is further supported by the afore-mentioned clinical data, where an improvement of functional iron status has been observed in response to weight loss (Tussing, *et al*, 2009).

Furthermore, one may speculate that pro-inflammatory cytokines interfere with erythropoietin production and also blunt the response of erythroid precursors to erythropoietin which is a well-recognized mechanism in the development of anemia of chronic disease and may thus also contribute to anemia in obese subjects (Nairz, *et al*, 2014). Rodent model suggest that a fat-rich diet may inhibit duodenal iron uptake via a hepcidin-independent mechanism (Sonnweber, *et al*, 2012). Moreover, the interaction between copper availability and iron homeostasis represents a potential link between dietary factors and iron uptake as low copper may lead to reduced ferroxidase activity necessary for iron export from enterocytes, macrophages and hepatocytes (Aigner, *et al*, 2008).

1-2-5 Relevance of ID to the course of obesity

Data is relatively scarce with regard to the relevance of ID to the course of obesity. However, several commonsense assumptions are indirectly supported by observational studies. ID and anemia may lead to fatigue and thereby to an additional decrease in physical activity, further aggravating weight gain (Munoz, *et al*, 2009). Additionally, ID may impair mitochondrial respiratory chain activity, thereby limiting exercise capacity and augmenting insulin resistance (Manios, *et*

al, 2013). Supporting this line of evidence, an improvement of several metabolic parameters was demonstrated with the correction of ID (Aktas, *et al*, 2014).

1-2-6 Response of iron parameters to weight reduction

Important data has been obtained from the study of changes in iron homeostasis in response to weight loss. Reported an improvement of iron status with unchanged serum ferritin concentrations and an increase in transferrin saturation after the intervention along with improvement of inflammatory markers (Gong, *et al*, 2014). Similarly, (Amato *et al* 2010) observed a decrease of serum hepcidin along with increased iron absorption after a six-month weight-loss program in children. (Amato, *et al*, 2010). However, no changes in iron status were observed in another study, though iron parameters were generally normal at baseline (Di Toro, *et al*, 1997). A very low-calorie diet even induced a further decrease in iron stores in obese children; however, their intervention performed does not represent a healthy, recommendable mode of weight loss. (Beard. *et al*, 1997). Insightful data have been derived from the observation of improvement of functional iron status as indicated by increased transferrin saturation and decreased serum hepcidin concentration in response to weight loss due to restrictive bariatric surgery (Tussing, *et al*, 2011). Largely, the prevailing evidence suggests that an unhealthy mode of weight loss in obese subjects is accompanied by an improvement of inflammatory markers along with re-established dietary iron absorption and serum iron concentrations as an indication for re-establishment of physiological iron homeostasis.

1-2-7 Previous Studies:

- **Clinical Data on Obesity-Related Iron Deficiency - Adolescence:**

Lower concentrations of serum iron with increasing BMI were observed several decades ago and confirmed in subsequent investigations. (Wenzel, *et al*, 1962). An analysis from the NHANES population found the risk for ID, defined as low transferrin saturation and low serum ferritin, to be twice as high in overweight adolescents compared to normal weight adolescents (Nead, *et al*, 2004), with similar results reported from an Israeli population (Pinhas-Hamiel, *et al*, 2003). Comparable results were documented in Iranian and Chinese investigations (Moayeri, *et al*, 2006). A further US study found a strong link between ID and BMI across all races, ages and amounts of dietary intake. (Tussing, *et al*, 2009). These studies unequivocally demonstrated lower serum iron availability with increasing AT mass in adolescents. Furthermore, iron uptake from the duodenum is limited in obese compared to normal weight children (Sanad, *et al*, 2011).

- **Adulthood**

In adults, several analyses demonstrated lower serum iron concentrations with higher BMI, particularly in women. Hence, one study reported lower serum iron concentrations in overweight women but no differences in males (Micozzi, *et al*, 1989).

No difference in serum iron between obese and normal weight were documented in another investigation (ozata, *etal* 2002). The association between adult obesity and low iron stores or anemia has been evaluated in a recent meta-analysis of all controlled studies (chengetal, 2012).

1-3 Rationale:

Both iron deficiency and obesity are signs of human malnutrition the correlation between both parameters are not well documented in Sudan. This could be attributed to lack of published research .The present study is an attempt to understand the possible correlation between obesity and iron deficiency among citizens of Khartoum State. Most donors of this study have visited Food & Nutrition Centre ,where this research was conducted, were claiming weight problems but not iron deficiency.

1-4 Objectives:

- **General Objective:**

assessment the obesity effect on iron profile in Khartoum state.

- **Specific Objective:**

- 1- To detect the obesity by calculation of BMI to differentiate between obese and non obese and determine the level of obesity of participants.
- 2- To measure serum iron, S. ferritin, TIBC, Transferrin in obese and non obese to determine the correlation between obesity and deficiency of iron profile.
- 3- To determine the level of obesity mostly affected by serum iron deficiency.

Chapter Two

Materials and Methods

2- Materials and Methods

2-1 Study design:

This study was conducted in the form of a cross sectional study.

2-2 Sampling:

Non-probability sampling method was used only for those who have been involved in sampling, then the blood analysis done to diagnose iron profile.

2-3 Study area:

Study was conducted at Food & Nutrition Centre–Scientist House Khartoum North.

2-4 Study population:

Study population include (49) obese and (51) non obese persons from both sex.

2-5 Inclusion criteria:

The obese and normal weight persons, residing in Khartoum state were free from underlying condition affecting iron profile level.

2-6 Exclusion criteria:

Those who had iron deficiency (anemic), were advised to take food supplements rich in iron. Any prevailing infections were excluded from the study.

2-7 Study duration:

This study was conducted during April -October 2015.

2-8 Specimen collection:

5ml of veins blood was taken by standard method on plain container serum prepared.

2-9 Specimen processing:

Sample was drawn to check for serum iron, serum ferritin, total iron binding capacity (TIBC) by biochemistry method.

2-10 Data collection:

The data was collected via, personal communications in form of standard questionnaire, and laboratory analytical procedures.

2-11 Iron profile

2-11-1 Serum ferritin:

2-11-1-1 Principle:

Serum ferritin causes agglutination of latex coated with antihuman ferritin antibodies'.The agglutination of latex particles is proportional to ferritin concentration and can be measured by turbidimetry

2-11-1-2 Reagents:

Reagent A: glycine buffer 170 mmol/L, sodiumazide 0.95g/L,PH8.2

Reagent B: reagent suspension of latex particle coated with antihuman ferritin anti bodies, sodium azide 0.95g/L

Ferritin standard: human serum concentration is given on the label.

The concentration value is traceable to the Biological Reference material WHO 94/572 (National Institute for Biological standard and Control, NIBSC).

2-11-1-3 Reference value:

Children: 7---140µg/L

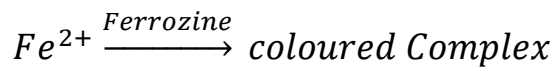
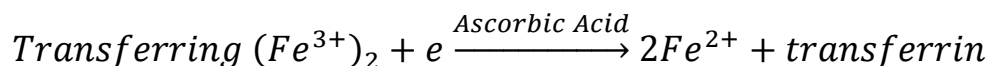
Men : 20 --- 250 µg/L

Women: 20 --- 200 µg/L

2-11-2 Serum Iron:

2-11-2-1 Principle:

The iron dissociated from transferring iron complex in weak acid medium. liberated iron is reduced into bivalent form by mean of ascorbic acid. Ferrousiron give with ferrozine a color complex. The intensity of the color formed is proportional to the iron concentration in the sample.



The absorbance is red at 562 nm

2-11-2-2 Reagent:

R1 Buffer	Acetate	100	mmol/L
R2 Reductant	Ascorbic	99.7	%
R3 Color	Ferrozine	40	mmol/L
Iron cal	Iron aqueous primary standard	100	µg/dl

2-11-2-3 Reference value:

Male : 65 --- 175 µg/dL = 11.6 --- 31.3 mmol/L

Female : 40 --- 160 µg/dL = 7.16 --- 26.85 mmol/L

2-11-3 Total iron binding capacity (TIBC) :

2-11-3-1 Principle :

Serum transferrin is saturated with an excess of Fe^{3+} and the unbound protein precipitated with magnesium carbonate. The total amount of iron was then determined. The difference between TIBC and initial serum iron (SI) yields the unsaturated iron binding capacity (UIBC).

2-11-3-2 Reagents:

R5	saturation solution	Iron solution	500µg/dL
R6	precipitating agent	Magnesium carbonate	

2-11-3-3 Reference value:

200 --- 400 µg/dL = 36 --- 72µmol/L

2-11-4 Transferring saturation:

TS are calculated from TIBC and SI by formula.

Transferring saturation % = (serum iron/TIBC) ×100

2-12 Data analysis:

Data was analyzed using computerized program Statistical Package for the Social Sciences (SPSS) one way anova.

2-13 Ethical clearance:

All the samples were collected after acceptance of the individuals under study.

Chapter three

Results

3- Results

The study included 100 adult male and female within age range from 18 to 70years old.

The case group enrolled (49) obese individual (49%) while control group include (51) individual within normal MBI (51%) match with case in age and living environment.

The case group report low mean level of s. iron (66.9 ± 35.4) vs (106.3 ± 35.4), s. ferritin (22.6 ± 19.5) vs (48 ± 30.5), transferrin saturation (26.4 ± 1.2) vs (40.6 ± 1.5).

Except the Total iron Binding Capacity report high level in case group when compared with control group (269 ± 58.3) vs (254 ± 56).

Age group (39-60) is more affected by obesity than others.

The course of obesity directly proportional with (s. iron, s.ferritin, T/S) reduction but TIBC is decrease when BMI is increase.

Overweight s.iron mean 82 ± 17 , s.ferritin 29.7 ± 13 , TIBC 272 ± 12 , T-S mean 31.8 ± 18

Obese s.iron mean 67.8 ± 25 , s.ferritin 24.9 ± 36 , TIBC 259 ± 37 , T-S mean 25.3 ± 16

Over obese s.iron mean 55.1 ± 17 , s.ferritin 21.7 ± 15 , TIBC 231 ± 11 , T-S mean 24.1 ± 18

Table No (2) :

Comparison of study variables between obese and non-obese

Parameters	No	obese	Total	Non obese	p.value*
Iron ($\mu\text{g/dL}$)	49	66.9 \pm 35.4	51	106.3 \pm 35.4	0.000
Ferritin($\mu\text{g/L}$)	49	22.6 \pm 19.5	51	48 \pm 30.5	0.000
TIBC ($\mu\text{g/dL}$)	49	269 \pm 58.3	51	254 \pm 5.6	0.193
Transferrin saturation %	49	26.4 \pm 1.2	51	40.6 \pm 1.5	0.000

Table No (3):

Effect of age on study variables

No	Parameters	18—38 Years	39—60 years	>60 years	p.value
1	Iron ($\mu\text{g/dL}$)	71.8 \pm 11	61 \pm 21	81.2 \pm 20	0.844
2	Ferritin($\mu\text{g/L}$)	25 \pm 15	20.6 \pm 31	22.5 \pm 19	0.15
3	TIBC($\mu\text{g/dL}$)	270.6 \pm 12	241 \pm 14	273.7 \pm 16	0.233
4	Transferrin saturation %	27.6 \pm 16	26.1 \pm 10	26.9 \pm 15	0.689

Table No (4):

Effect of obesity course among study variables

NO	Parameters	Over weight	obese	over obese	p.value
1	Iron($\mu\text{g/dL}$)	82 ± 17	67.8 ± 25	55.1 ± 17	0.000
2	Ferritin($\mu\text{g/L}$)	29.7 ± 13	24.9 ± 36	21.7 ± 15	0.002
3	TIBC($\mu\text{g/dL}$)	272 ± 12	259 ± 37	231 ± 11	0.801
4	Transferrin saturation %	31.8 ± 18	25.3 ± 16	24.1 ± 18	0.000

Chapter Four

Discussion, conclusion and
recommendations

4- Discussion, conclusion and recommendations

4-1 Discussion

This study was done to assessment of the effect of obesity on iron profile in Khartoum state Residents.

The obese group composed of 49 (forty nine) obese individuals the level of obesity was determined by calculation of BMI.

Non-obese group composed of 51(fifty one) normal weight individuals matched with case group in age, living environment and feeding habits..

The study participant fell within age range 18 to 70 years old (mean age 37 ± 14).

Among the case group there was a peak incidence of obesity age range 39 to 60.

The reduction of, S.Iron, S.ferritin, TIBC, S.T/S was observed in Overweight s.iron mean 82 ± 17 , s.ferritin 29.7 ± 13 , TIBC 272 ± 12 , T-S mean 31.8 ± 18 , Obese s.iron mean 67.8 ± 25 , s.ferritin 24.9 ± 36 , TIBC 259 ± 37 , T-S mean 25.3 ± 16 , Over obese s.iron mean 55.1 ± 17 , s.ferritin 21.7 ± 15 , TIBC 231 ± 11 , T-S mean 24.1 ± 18 .

Normal weight group s.iron mean 106.3 ± 35.4 , s.ferritin 48 ± 30.5 , TIBC 254 ± 5.6 , T-S mean 40.6 ± 1.5 .

The above cited results reveal that the level of obesity is playing a major role in reduction of iron profile.

a similar result has been documented in US Health and Nutrition Examination Survey (NHANESI) to characterize these association (Micozzi, *et al*, 1989).

4-2 Conclusions

- Abnormal parameters of iron status may lead to iron deficiency anemia are frequently findings in over weight and obese subject.
- Impaired functional iron status is mainly link to adipostissue inflammation and increased expression of the systemic iron regulatory protein hepcidin.
- Cytokines such as $\text{TNT-}\alpha$, IL-1 and IL-6 along with adipokines (leptin, resistin) or hepcidin may represent signal from obese, inflamed AT facilitating change in physiological iron homeostasis.

4-3. Recommendations

- 1- Screening for ID should be considered for individual with elevated BMI.
- 2- The determination of sTfR might be a useful tool to show the relationship between tissue iron concentration and obesity.
- 3- Increasing awareness of the importance of physical activity and carrying out nutritional education programs are required.
- 4- Owing to its underlying mechanism of impaired iron absorption via the gut, treatment of iron deficiency by oral supplementation is frequently insufficient, and parenteral substitution is thus necessary.

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Appendix

Appendix (1): Questionnaire:

Name ID.....

Age Years

Residence

Course of obesity

Normal weight over weight Obese Over obese.....

Presence of chronic disease: Yes () No ()

Use of iron supplement: Yes () NO()

Iron profile:

Serum iron $\mu\text{g/dl}$

Serum ferritin $\mu\text{g/l}$

TIBC: $\mu\text{g/dl}$

Transferrin saturation:%

Appendix (2):spectrophotometer

