AMERICAN UNIVERSITY OF BEIRUT

THE RELATION BETWEEN THE TYPE OF ADIPOSITY, INFLAMMATION, AND IRON ABSORPTION IN PRE-MENOPAUSAL WOMEN

by
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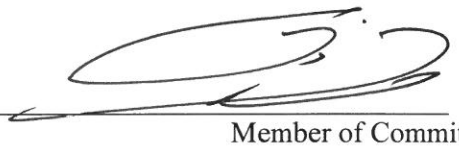

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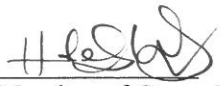

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AN ABSTRACT OF THE THESIS OF

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Iron deficiency is one of the most common deficiencies that people face. Many reasons are found to be behind it, of which is the prominent obesity factor. Known as a state of low-grade inflammation, obesity has been proved to increase the release of inflammatory cytokines and hepcidin, a hepato-protein, which negatively affects the absorption of ingested iron. On the longer run, if untreated, iron deficiency leads to anemia.

Globally, none of the previously conducted studies has focused on the location of adiposity and its effect on iron absorption to the best of the investigator's knowledge. In Lebanon, this study aims to examine whether it is the absolute amount of fat or the location of fat, leading to an inflammatory state, is responsible for the decrease in iron absorption in females of childbearing age.

A total of 118 premenopausal women divided into 4 categories (lean, overweight, obese class I, and obese class II) were recruited. Anthropometric measurements and body composition using DXA were collected. Iron absorption was determined using three blood samples collected at baseline (overnight fasted), 2 weeks post the ingestion of a labeled 57Fe load, and 2 hours following the ingestion of glucose/sodium ferrous citrate load. They were analyzed for iron levels (serum iron, transferrin receptors, transferrin saturation, total iron binding capacity, ferritin, hepcidin, hemoglobin, mean corpuscular volume and red cell distribution width), fractional iron absorption and inflammatory parameters (CRP, AGP, hepcidin and ferritin).

In the wake of the literature gap on the relation between adiposity location and iron metabolism, this study would provide science with further clarification that allows healthcare professionals to categorize obese and overweight individuals according to their visceral adiposity, having the latter in mind while prescribing their iron medical treatment.

The results of this study showed that markers for iron status were not affected by the change in BMI (p-value>0.05). Moreover, fractional iron absorption remained insignificantly change despite of the transition to a higher BMI category (p-value=0.227). As for body composition markers, they significantly affected the levels of AGP & CRP yet ferritin and hepcidin levels were indifferent when those markers were altered. As for fractional iron absorption, it was affected by the change in body composition of the participant, and the change in markers of iron status. Participants of this study had a higher baseline adiposity compared to previously done studies, lower baseline iron status, and a higher inflammatory profile. Only body iron stores remained the significant factor affecting fractional iron absorption (p-value=0.014).

Neither the absolute value of the fat mass in a female body nor the different fat

location showed significant results on iron absorption in this study. Moreover, the low-grade inflammation observed didn't significantly iron status. Further studies are required to be done on this topic yet applied on metabolically-unhealthy individuals in order to see if the effect of the previously mentioned markers will be different or it will remain insignificant.

CONTENTS

ILLUSTRATIONS

TABLES

ABBREVIATIONS

%	Per Cent
/	Per
&	And
±	Plus or Minus
=	Equal
<	Less than
>	Greater than
≤	Less than or equal
≥	Greater than or equal
A/G	Android/Gynoid Ratio
ACD	Anemia of chronic disease
AdipoR2	Adiponectin receptor 2
AGP	Alpha (1)-acid glycoprotein
ALA-S	Aminolevulinic-acid synthase
AMPK	Adenosine monophosphate kinase
ANOVA	Analysis of variance
ATM	Adipose tissue macrophages
BAT	Brown adipose tissue
BMI	Body mass index
BW	Body weight
CBC	Complete blood count
CDC	Centers for Disease Control and Prevention
CHD	Coronary heart disease
CI	Confidence interval

Ci	Crowding index
Cm	Centimeter
CO2	Carbon dioxide
CRP	C-reactive protein
Cu	Copper
d	Day
dl	Deciliter
Dcytb	Duodenal cytochrome b
DEXA	Dual-energy X-ray Absorptiometry
DMT1	Divalent metal transporter 1
et al.	And Others
EDTA	Ethylenediaminetetraacetic acid
ETC	Electron transport chain
FFA	Free fatty acids
FDA	Food and Drug Administration
Fe	Iron
FER	Ferritin
FeSO4	Ferrous sulfate
Fl	Fluid
FMNH2	Riboflavin
FP-1	Ferroportin-1
g	gram
GI	Gastrointestinal
GLUT4	Glucose transporter type 4
Hb	Hemoglobin
HbA1c	Hemoglobin A1c
HCL	Hydrochloride

HDL	High density lipoprotein
HSL	Hormone sensitive lipase
Ht	Height
HTN	Hypertension
IL-1	Interleukin 1
IL-6	Interleukin 6
IR	Insulin resistance
IRE	Iron responsive element
IRE-BP	Iron responsive element binding protein
IRS	Insulin receptors substrates
JNK-1	Jun N-terminal kinase-1
Kg	Kilogram
L	Liter
LDL	Low density lipoprotein
LPL	Lipoprotein lipase
LPS	Lipopolysaccharide
m	Meter
MCV	Mean corpuscular volume
mg	Milligrams
mol	mol
n	Number
NADH	Niacin
NCDs	Non-communicable diseases
NIDDM	Non-insulin dependent diabetes mellitus
NO	Nitric oxide
NOD	Nucleotide oligomerization domain
NOD1	Nucleotide oligomerization domain 1

NOD2	Nucleotide oligomerization domain 2
O2	Oxygen
OIAT	Oral iron absorption test
OGTT	Oral glucose tolerance test
P	P value
pH	Acidity scale
PI3K	Phosphoinositol-3 kinase
R	Pearson’s correlation
R2	R Squared
RBC	Red blood cells
RDA	Recommended dietary allowance
RDW	Red cell distribution width
RE	Reticulo-endothelial
ROS	Reactive oxygen species
sTfR	Serum transferrin receptors
SAT	Subcutaneous Adipose tissue
SD	Standard Deviation
SE	Standard Error
SFA	Saturated fatty acids
SPSS	Statistical Package for Social Sciences
T0	Time zero
T1	Time after 2 hours
T2DM	Type 2 diabetes mellitus
TFA	Trans fatty acids
TfR	Transferrin receptors
TfR2	Transferrin receptor 2
TIMS	Thermal ionization mass spectrometry

TG	Triglycerides
TLR	Toll-like receptor
TLR2	Toll-like receptor 2
TLR4	Toll-like receptor 4
TNF	Tumor necrosis factor
µg	Microgram
UL	Upper limit
USDA	Unites States Department of Agriculture
VAT	Visceral Adipose Tissue
VLDL	Very low density lipoprotein
vs.	Versus
WAT	White adipose tissue
WHO	World Health Organization
Wt	Weight
yrs	years

To my idol, my mother

To My Best Friends
Nour El Hamwi
Racha Youssef
Sirine Baghdadi

For always standing by me

CHAPTER I

INTRODUCTION

Being the most frequent deficiency, iron deficiency (ID) affects around 30% of Earth's population (Sal *et al*., 2018). On the long run, untreated ID would develop into IDA. Considered as a worldwide public health concern, IDA has various detrimental side effects on an individual's health depending on their age or gender: In individuals below 18 years of age (children & adolescents), motor dysfunctions and mental compromises can result (Haas & Brownlie, 2001). In children, visual, mental and auditory troubles are witnessed. Even before reaching the phase of clinically-diagnosed IDA, ID has led females to suffer from cognitive difficulties in adolescence and chronic fatigue in adulthood (Algarin *et al*., 2003; Verdon *et al*., 2003). Numerous physiological disturbances can cause ID of which is obesity. ID is known to be one of the most common outcomes of this low-grade inflammatory status of obesity (Sal *et al*., 2018).

The health-consequences of obesity were the reason why many scientists have focused on all the mechanisms related to obesity, how it promotes inflammation, insulin resistance, its link to ID & IDA in their studies while trying to propose potential therapeutic measures to cease this disadvantageous trend.

Several studies have been conducted on how obesity promotes a high inflammatory profile that is the root for arising insulin resistance that is seen to be a crucial factor for the development of type-2 diabetes mellitus (T2DM). It has been justified that obesity puts the human body under a chronic low-grade inflammation marked by elevated levels of C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and IL-1 in plasma (Singer & Lumeng, 2017). The

previous pro-inflammatory cytokines act differently towards a common goal which is directly to increase inflammation in the body and indirectly to worsen insulin resistance.

It has been a continuous effort to well examine the effect that inflammation and insulin resistance have on iron deficiency and ultimately anemia. New mechanisms emerged over the years, introducing the previously-inexistent hepcidin compound.

Although many earlier studies have focused on obesity in general, the approach of distinguishing between central and peripheral obesity more recent. Fat in the body is divided into two categories: subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). The latter is more metabolically active, releasing free fatty acids (FFAs) at a higher rate, has lower proliferation rate which is why its expansion happens mostly by hypertrophy compared to hyperplasia in SAT. This explains why VAT has been seen and treated as the more harmful type of fat by researchers and a decrease in VAT levels in the human body is of higher priority in obese people since it influences the risk of metabolic obesity-related diseases like insulin resistance, hypertension, diabetes mellitus and cardiovascular disorders (Neeland *et al*., 2015; Chandra *et al*., 2014; Neeland *et al*., 2012).

Plentiful amount of studies was published on the mechanisms linking obesity to insulin resistance and inflammation. Furthermore, other ones were also done on how insulin resistance and inflammation affect iron metabolism.

Yet, scarce data is available on how the different fat locations in the human body can have diverse results on specific minerals metabolism in the human body and how they affect homeostasis. Moreover, to the best of our knowledge, no former study has been conducted on how visceral obesity, inflammation and insulin resistance can affect iron overall metabolism thus its subsequent IDA occurrence.

This type of study is highly prominent since healthcare practitioners have been

treated IDA in all types of obese people similarly: the prescription of oral iron supplements. This has been shown to be ineffective yet potentially harmful in people with central/visceral type of obesity since due to their high level of VAT thus high hepcidin levels, iron absorption is diminished (Schmidt, 2015). Thus, prescribing oral supplements, commonly ferrous sulfate ($FeSO_4$) according to Moretti *et al*., for all obese patients as a method to treat obesity-related IDA doesn't seem to be the ideal treatment (2015). The unabsorbed iron fraction would lead to gastrointestinal (GI) distress, nausea and vomiting post ingestion, which typically hit about 6-12% of people on oral supplements (Saha, 2007; Adamson, 2001). The more common symptoms are diarrhea and constipation which tend to affect approximately 6% of the people on oral treatment. Some studies suggest that those side effects occur since the unabsorbed iron remains in the intestinal lumen, other ones propose that they are due to the free radicals generated by the free pro-oxidant non-transferrin-bound iron (Nolte *et al*., 2011). The latter would favor the development of oxidative stress in the GI tract. One addition proposed side effect is that unabsorbed iron, known as a primary feed for pathogens and microbes, would be a supporting factor for their adverse, health-jeopardizing growth and proliferation (Schümann *et al*., 2007).

Moreover, to examine the absorption level of ingested oral iron in people with high visceral fat, who are suggested to have decreased iron absorption at the intestinal level, iron stable isotope Fe^{57} is used as an oral supplement to trace its metabolic path after ingestion. The latter is being used as a method to test the absorption rates in people with various disorders. According to International Atomic Energy Agency, since most of the absorbed iron gets incorporated in the human body into the erythrocytes at the level of the bone marrow, iron absorption in individuals can be tested using the isotopic iron enrichment in erythrocytes which is typically conducted 14 days after the ingestion

(2012; Moretti *et al*., 2015).

In addition, it is important to mention here that this gold standard method of measuring iron absorption is not the most practical method because it's expensive, requires high technical skills and the stable iron isotope is hardly accessible (IAEA, 2012). Several studies have tried to come up with alternative approaches to measure iron absorption in a more cost-effective, practical, easy-to-perform and all-time available approach. Stable iron isotope studies are the gold standard to monitor iron absorption and incorporation into red blood cells using thermal ionization mass spectrometry (TIMS) (Turnlund, 1987). The other method that can be used is the oral iron absorption test (OIAT), which is a simple test consisting of an oral iron load preceded and followed by a blood test to determine the rise in plasma iron concentration as a measure of intestinal iron absorption. This method is faster and easier than the labeled isotope, but has not been compared to that of stable isotope for validation (Kobune *et al*., 2011; Santarpia *et al*., 2012).

As a final point, the main objective of this study is to examine the effect of visceral adiposity, inflammation and insulin resistance on iron absorption in overweight and obese pre-menopausal women. A second objective in this study is to determine the comparability of the two methods by which iron will be measured: Oral iron absorption test (OIAT) and iron stable isotope enrichment in red blood cells.

This thesis entails 6 chapters. The following chapter is the literature review which will present all the background information related to iron metabolism, different types of iron and its metabolism, and how obesity causes it in addition to other health consequences related to both obesity and iron deficiency including insulin resistance and inflammation. Chapter II addresses the materials and methods and will describe the study design and all the details related to data collection, laboratory and statistical

analyses. Our results and their interpretation and discussion are presented in Chapters III and IV respectively. Chapter IV contains comparisons of our results as well with diverse results obtained in other studies and discusses our study's limitations and strengths. Finally, the final chapter reviews the findings of this study, summarizes its main points and gives a brief conclusion.

CHAPTER II

LITERATURE REVIEW

This chapter begins by discussing several aspects related to the mineral iron: its metabolism, the importance of iron in the homeostasis of the human body, iron-deficiency and iron-related diseases, and the reasons behind them, like obesity. Furthermore, background information is given about obesity: prevalence, definition, physiology, and its health-related disorders. The last section of this chapter reviews the mechanisms of insulin resistance and high inflammatory profile are discussed and linked to obesity and iron status.

A. Background Information

Anemia is a condition that touches about 30% of people, of which around 50% is caused due to iron deficiency. It affects individuals of different age groups, starting from children to elderly. The factors causing its occurrence are more specific to the age group, but they all reach the same end: Iron-deficiency anemia (IDA). Iron deficiency comes in types: absolute iron deficiency and functional iron deficiency. The difference between those two is that absolute iron deficiency is characterized with low or depleted iron stores in the human body whereas functional iron deficiency takes place when iron stores in the body are within the normal range yet the defect is in the supply of iron to the bone marrow which will lead to hindered all iron-related functional processed in the body (Lopez *et al*., 2016). Certain age groups are considered of high risk, for instance: children below 5 years of age, women of childbearing age and pregnant females. They have a higher chance of developing iron deficiency and furthermore if not treated, IDA.

Before going deeper into anemia, it is important to give more details about this essential mineral, iron, that is behind it all.

B. Iron Metabolism

Iron is a transitional metal with a molecular weight of 55.8g/mol. It is the second most abundant metal in the earth's crust after aluminum. It exists in several oxidation states (Fe^{6+} to Fe^{2+}). The two stable oxidation states present in human body and food are Fe^{3+} and Fe^{2+}. It is involved in many oxidation-reduction reactions to mention photosynthesis, Kreb's cycle, electron transport chain, O_2 and CO_2 transport. It is an important trace element because globally, it is the most common deficient nutrient especially in young girls. If not treated, iron deficiency on the long run would lead to IDA.

Iron in the diet comes in two forms: Non-heme iron and Heme iron. On average, the western diet contains around 5-7mg of iron per 1000Kcal.

The *non-Heme* iron is the inorganic form of iron that is found either in plant foods or in milk and its derivatives as lactoferrin or in eggs bound to a phosphate group. It comprises both the ferrous (Fe^{2+}) and the ferric form (Fe^{3+}) with the latter being the most common. Ferric iron is the oxidized stable form of iron that has lost an electron by the process of oxidation. In foods, 99% of the iron content comes in the ferric form. It is important to mention that a large number of enzymes contain non-heme iron.

As for the *Heme iron*, 50-60% of iron is in meat, fish and poultry. It comes as a part of a complex heme group. It has several functions:

- *Hemoglobin*: Hemoglobin is a protein found in red blood cells containing Heme groups. The Heme content of hemoglobin allows it to have a role in oxygen transport around the human body.

- *Myoglobin:* Another protein found in muscles of the human body. It is responsible for the oxygen reserve similarly to hemoglobin.
- *Enzymes & Energy Metabolism:* Heme iron is also found in enzymes and in transport proteins in the electron transport chain (ETC). Due to its presence in some cytochromes of the ETC, it is considered to have a role in energy metabolism.

1. Distribution in the Human Body

Iron is widely distributed in the human body. In the duodenum, the average amount of consumed iron is 1-2mg per day. The total mass of iron found in plasma transferrin is 3mg. In the circulating erythrocytes, hemoglobin holds around 1800mg of iron where myoglobin contains around 300 mgs. In the liver, there is storage of 1g of iron and in the reticulo-endothelial macrophages, around 600mg of iron are found. Around 1-2mg of iron is lost per day due to sloughed mucosal cells, desquamation, menstruation and other blood losses. Women store less in iron in their liver yet iron stores in the liver is a prerequisite for a successful pregnancy.

2. Iron Digestion & Absorption

a. Heme Iron

Proteases hydrolyze the globin of hemoglobin and myoglobin. The absorption of Heme iron by the mucosal cells depends on the body iron stores. Heme-iron binds to Heme Carrier-Protein-1 (HCP-1) at the brush border side of the mucocyte before going inside it. Inside the mucosal cell, it is transported through the cell by diffusion, binding to histidine or cysteine, or binding to mobilferrin and converted to ferritin and based on the need of the human body, can be used by the mucosal cell itself as a cofactor for enzymes, released into the circulation for peripheral cell use or stored as apoferritin

inside the mucosal cell for further cell use. To leave the mucosal cell, under the action of heme oxygenase, hephaestin converts Fe^{2+} to Fe^{3+} which allows Fe^{2+} to escape through ferroportin 1 (FP1) before it binds to transferrin and goes into the circulation. If the person has normal iron status, the absorption is only up to 15% whereas in situations of deficiencies, iron absorption is up to 35%. The absorption of iron occurs most efficiently in the duodenum.

b. Non-Heme Iron

At the level of the stomach, HCL and pepsin play a role in providing iron solubility and releasing it from foods. HCL converts Fe^{3+} to Fe^{2+} which remains soluble up to a pH of 8. Once in the proximal small intestine, the medium becomes alkaline. Some Fe^{2+} is oxidized to Fe^{3+}, another part forms an insoluble complex of ferric hydroxide, and the remaining iron chelates with ligands. The process of Fe^{3+} chelation improves its solubility yet only Fe^{3+} is absorbed into the enterocytes. At the brush border site of the intestinal cells, duodenal cytochrome b (Dcytb) i.e ferric reductase binds to Fe^{3+} and converts it to Fe^{2+} where divalent metal transporter 1 (DMT1) allows for its entry inside the mucosal cell. Considering that the iron might come from a dairy source, lactoferrin will bind to lactoferrin receptors at the same side of the mucosa cells mediating Fe entry inside the mucosal cells. Having a common destination with the consumed Heme-Fe, ferritin is obtained and the same pathway follows.

The mechanism behinds this remains unclear yet it is thought that integrin, a membrane protein, facilitates this absorption. Fe^{2+} is better absorbed than Fe^{3+} where the former binds to receptors on the brush border side of the mucosal cells for absorption. It is important to mention here that the non-heme iron absorption ranges between 2% to 10%. The amount of DMT-1 and FP-1 on both sides of the mucosal

cells, along with the amount of Fe stores inside the hepatic cells and the number of transferring receptor 2 (TfR2) on its surface, communicates with Fe sensing property of the hepatocyte which, accordingly, changes hepcidin expression and release into the circulation and ultimately Fe absorption at the level of the small intestine.

3. Iron Transport

Iron, as Fe^{2+}, is converted to Fe^{3+} by ceruloplasmin-Cu^{2+} before it binds to the hepatically-made transferrin. Other transporters like lactoferrin, ferritin and extracellular low-molecular-weight species can also carry iron in the circulation. It is important to have it bound to a carrier because unbound iron can be used by bacteria or generate free radicals, where both cases are not beneficial, rather harmful, to the human body.

a. <u>The Transferrin Cycle</u>

When transferrin-bound iron reaches the peripheral cells, it binds to transferrin receptors on the cell membrane. The complex moves into the cytoplasm of the peripheral cell via endocytosis and forming an endosome. Inside the endosome, the pH is decreased to 5.5 to release iron from transferrin which would lead to Fe release into the cell cytoplasm through the endosomal membrane. Any decrease in the concentration of the intracellular iron would lead to a positive feedback on the transcription process of the genes encoding for transferrin receptors.

4. Iron Storage

Iron is primarily stored in the liver where the amount of hepatic iron which accounts for 60% of all iron in the body. The remaining 40% are distributed among the reticuloendothelial (RE) cells in the spleen and bone marrow. The main storage form of

iron in cells is ferritin, accounting for up to 20% among all other forms.

Ferritin can be synthesized in the liver, spleen, bone marrow in addition to the intestines and can store up to 4,500 atoms of iron. It is in constant degradation and re-synthesis process. It is an acute-phase reactant whose concentrations can be affected by conditions of infection or inflammation.

Other than ferritin, hemosiderin is another form of iron storage which holds up to 35% of total body iron and is considered as a degradation product of ferritin being the pathway used by the body when ferritin levels are too high.

To release iron from its storage forms, whether ferritin or hemosiderin, it requires several cofactors: Riboflavin ($FMNH_2$), niacin (NADH), vitamin C and glutathione or cysteine.

5. Iron Excretion

Iron homeostasis is hugely controlled at the level of absorption more than excretion. Adult men excrete around 0.9-1.05mg of Fe/day as follows: 0.6mg are excreted at the level of the GI system, 0.2-0.3mg at the level of the skin and 0.1mg by urine.

As for females, 35-80ml of blood is lost during menstruation, which accounts for 0.6-0.7mg of Fe/day. Other cases such as bleeding or blood donation are also considered as situations of iron loss whereas cases of pregnancy and lactation are approached as situations of increased iron use by the body therefore increased needs of body iron.

6. Iron Turnover and Cell Regulation

a. Iron Turnover

Iron turnover is mainly regulated by hemoglobin degradation. After a life lasting for 120 days, erythrocytes are captured and catabolized by the macrophages in the spleen into bilirubin.

b. Cell Regulation

Iron regulation in the cells depends on three factors: the amount of apoferritin in the cytosol, the number of transferrin receptors on the cell membrane, and the amount of Gamma-aminolevulinic acid synthase (ALA-S) in the mitochondria.

When the iron cellular content is low, Iron Responsive Elements Binding Proteins (IRE-BP), a post-translational regulatory protein, have higher affinity to bind to the Iron Responsive Elements (IRE) found in the untranslated region of the mRNA encoding for both ferritin and transferrin receptor. The low iron availability situation allows for the ferritin mRNA translation to be repressed while the transferrin receptor mRNA translation to be enhanced.

However, when iron levels in the cell are elevated, the affinity of the IRE-BP to bind to IRE is lower which leaves the mRNA translation of ferritin stabilized while the translation of the TfR repressed.

7. Effect of Diet

Obesity develops as a result of the consumption of unbalanced meals. The low-cost fast foods are highly prevalent in refined carbohydrates and fats while they lack enough essential nutrients, particularly, iron (Seltzer *et al*., 1963).

Furthermore, other than the low intake of iron due to the trendy consumption of

fast foods, other studies have focused on the intake of heme and non-heme iron on serum iron levels in obese people. Those studies show that the intake of either heme or non-heme iron as well as the presence of iron chelators in the diet showed little effect on obese people's serum iron level (Menzie *et al*., 2008).

a. Dietary Factors Affecting Heme Fe Absorption

- *Amount of Heme Fe in meal:*

The absorption of Heme-iron declines to 2-3% if the iron is not given with a meal containing meat.

- *Calcium content of the meal:*

Calcium whether found in salts or dairy products, inhibits both Heme and non-Heme iron absorption. For instance, the consumption of 165mg of calcium, which is found in one cup of milk, decreases iron absorption by 50%.

- *Food preparation:*

High temperature cooking would convert Heme-iron to non-Heme iron which is the form that is less bioavailable thus decreasing iron absorption by the human body.

b. Dietary Enhancers of Non-Heme Fe Absorption

- Organic acids like ascorbic, citric, malic, lactic and tartaric acids in addition to taurcholic acids improve non-Heme iron absorption.
- Sugars like fructose and sorbitol, product of animal protein digestion (cysteine-containing actin and myosin) would as well enhance it.

c. Dietary Inhibitors of Non-Heme Fe Absorption

- Polyphenols like tannins in tea (around 60%) and coffee (around 40%);

oxalates in spinach, berries, chocolate and tea; phytates in maize and whole grain would all reduce non-heme iron absorption.

- Zinc-containing and manganese-containing foods also have an effect on absorption since both Zn and Mn compete with Fe on the same absorptive pathway.
- Calcium and phosphorus chelate iron at the intestinal mucosa by forming a complex.
- Soya protein, the preservative EDTA, phosvitin in egg yolks also interfere.

It is important to mention that, in cases of iron deficiency, the absorption of Heme iron is less affected than non-Heme iron.

8. Symptoms of Iron Deficiency

When iron deficiency takes place, several symptoms occur. If left untreated, the iron deficiency progresses into IDA. Pica, impaired thermoregulation (due to impaired thyroid function), impaired immune function, impaired mental function (low attention span, low intelligence scores, and perceptual disturbance), impaired physical performance (lethargy and apathy), glossitis, angular stomatitis, koilonychia, fatigue, blue sclera, altered drug metabolism, increased absorption of heavy metals like lead and cadmium, increased insulin sensitivity and complications of pregnancy (premature delivery, low birth weight and infant morbidity) are all possible scenarios resulting from iron deficiency.

9. Stages of Iron Deficiency

a. Iron Depletion

The first stage of iron deficiency is labeled as iron depletion. A decrease in iron stores occurs and is manifested physiologically by a decrease in serum ferritin levels.

b. Iron-Deficient Erythropoeisis

After stage I, the stores are fully depleted and the human body is not absorbing enough iron from the diet to replenish the ferritin stores and to provide the iron body need to fulfill its metabolic functions which leads to impaired hemoglobin synthesis.

c. Iron Deficiency Anemia

After having hemoglobin synthesis impaired, the clinical manifestation of this stage is reduced hemoglobin concentrations.

10. Iron Requirements

Iron is required mostly during periods of growth, pregnancy and lactation:

Life Stage Group	**Females** *RDA (mg/d)*	**Males** *RDA (mg/d)*	**UL** *(mg/d)*
	Infants		
0-6 months	0.27	0.27	40
7-12 months	11	11	40
	Children		
1-8 yrs	7	7	40
9-13 yrs	8	8	40
14-18 yrs	15	11	45
19-50 yrs	18	8	45
>51 yrs	8	8	45
	Pregnancy		
< and>18 yrs	27	-	45
	Lactation		
<18 yrs	10	-	45
>19 yrs	9	-	45

* Dietary reference intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington DC: National Academy Press. (2001)

Laboratory Measurements Commonly Used in the Evaluation of Iron Status:

Stages of Iron Deficiency	Indicator	Diagnostic Range
Depleted stores	- Stainable bone marrow iron - TIBC - Serum ferritin concentration	- Absent - >400ug/dl - <12ug/dl
Early functional iron deficiency	- Transferrin Saturation - Free erythrocyte protoporphyrin - Serum TfR	- <16% - >70ug/dl erythrocyte - >8.5mg/L
IDA	- Hb concentration - Mean cell volume	- <130g/L (male) - <120g/L(female) - <80Fl

Conditions Associated with Iron Deficiency Anemia:

Physiological		-Infants -Adolescent girls -Pregnant females -Elite Athletes
Pathological	*Blood Loss*	-Digestive tract: colon cancer, gastric cancer, inflammatory bowel diseases, ulcers, parasites -Gynecological blood loss -Surgery -Blood in urine, pus in blood -Hemodialysis -Non-Steroidal Anti-inflammatory drugs, aspirin
	Malabsorption	-Celiac disease -Gastrectomy -Helicobacter pylori -Gut resection, atrophic gastritis, gastric bypass, bacterial overgrowth -Nutrient-nutrient interaction -Pica syndrome -Proton pump inhibitors and H_2 antagonists
	Anemia of Chronic Disease	-Chronic Heart Failure -Cancer -Chronic Kidney disease -Rheumatoid arthritis -Obesity -Inflammatory bowel disease
	Genetic Disorders	-Iron-refractory iron-deficiency anemia -Others: DMT-1 deficiency anemia, Fanconi anemia, Pyruvate kinase deficiency

Source: Lopez, A., Cacoub, P., Macdougall, I.C. & Peyrin-Biroulet, L. (2016). "Iron deficiency anaemia". *The Lancet*, *387*(10021), 907-916.

As the above table shows, many reasons are behind the development of iron deficiency. One of those factors is the prominent ‘obesity factor’. It is important to go into further details of how obesity affects iron metabolism and homeostasis as well as the other detrimental side effects it has on human health and wellbeing.

C. Obesity

Worldwide, the two most prevalent nutritional disorders are obesity and iron deficiency (Harris, 2004). In children, the consequences of iron deficiency are not limited to anemia but they also include impairment in children’s cognitive function, delayed development and learning abilities (Halterman *et al*., 2001). As per the National Health and Nutrition Examination Survey (NHANES), as BMI increases the risk of developing iron deficiency increases well (Nead *et al*., 2004). Several causes have been discussed to justify this association.

1. Prevalence Worldwide & MENA Region

Obesity, being a public concern & phenomenon, has been tremendously increasing during the past decades. According to the World Health Organization (WHO), worldwide obesity has approximately reached the triple of its prevalence since 1975. In 2016, around 1.9 billion adults, aging above 18 years, are overweight. Among those 1.9 billion, 650 million are obese (2017) (http://www.who.int/mediacentre/factsheets/fs311/en/). Whether high-income countries or low-income ones, obesity has reached critical levels imperiling the chances of those countries of being capable to handle the rising treatment costs of obesity-related diseases (Hossain *et al*., 2007).

Narrowing it down to the Eastern Mediterranean region, the scenario is closely similar. A study has mentioned that in Arabian Gulf states, 66-75% of adults and 25-

40% of children and adolescents are either overweight or obese (Ng *et al*., 2010). Lebanon, as a typical Eastern Mediterranean country, lacks studies on obesity trends. According to a national-based cross-sectional study conducted in 2003, the prevalence rate of overweight was 57.7% among adult men and women (> or =20 years) while obesity hit 49.4% in a sample of 2104 individuals (Sibai *et al*., 2003).

2. Definition

An individual is classified, by definition, as overweight or obese when excessive adipose tissues accumulate in the body leading to potential harm to one's health. It is measured by the Body-Mass Index (BMI) where a BMI between 18.5kg/m^2 and 25kg/m^2 puts the individual in the normal-weight range whereas an individual with a BMI greater than 25kg/m^2 is considered as an overweight case and a BMI greater than 30kg/m^2 is considered as obese (WHO, 2017).

3. Causes of Obesity

Due to its alarming consequences, obesity has been occupying the primary interest and closest attention of researchers and scientists. It has been defined as the result of an interaction between genetics and environmental factors (Pi-Sunyer, 2002). The latter will be described in this section:

a. Genetics

One factor contributing to the high prevalence of obesity is genetics. It contributes to 30-40% of those observed changes in individuals' BMI whereas the environmental surrounding those people are subject to, is responsible for 60-70% of the resulting problem. The effect of genetics on its own has been proven in several studies.

Bouchard *et al*. studied the effect of long-term overfeeding on different pairs of monozygotic twins given the same diet over a 100-day period (1990). The weight gain that was observed differed among pairs of twins, yet the difference tremendously decreased between each two twins of the same pair who showed similarities in body weight, fat mass, percentage of fat and estimated subcutaneous fat (Bouchard *et al*., 1990). These results, like many others, show that despite the environmental factors that individuals might be subject to; there is always an underlying genetical factor that is capable of being in the obesity equation and is able to change the end-result accordingly.

b. Environmental Factors

However, genetics are not the only ones to blame. The environmental factors a person is subject to might trigger the expression of the specific set of genes which previously made him predisposed to obesity. In fact, other studies focused on the effect of environmental factors solely regardless of the genetic predisposition of individuals to eliminate the effect of possible confounding factors thus being able to clearly assess the role a person's environment plays. Populations having the same genetical pool were studied while having different lifestyles (Ravussin *et al*., 1994). The findings show that although both groups are subject to the same genetic predisposition, the ones traditional lifestyle that is characterized by a higher physical labor, lower animal fat consumption and more complex carbohydrates in their diets present less prevalence of obesity, lower chances of cardiovascular diseases and non-insulin-dependent diabetes mellitus (NIDDM) (Ravussin *et al*., 1994). Thus, even if an individual is genetically predisposed to obesity, it is crucial to know that it is not the outcome of genetics alone, nor environmental factors alone but the interaction between those two factors.

4. Consequences of Obesity

The reason behind the great worries about obesity, its causes and prevalence are also due to the consequences it leads to.

According to WHO, a high BMI has been treated as and proven to be a major risk factor for non-communicable diseases (NCDs) (2017). The spectrum of weight-related NCDs includes cardiovascular diseases mainly stroke & heart diseases which led the list of causes of death in 2012. To add, diabetes, musculoskeletal disorders, and osteoarthritis specifically are also inclusive (WHO, 2017). Other diseases that have been associated with obesity are the following: Insulin resistance and hyperinsulinemia, Type II Diabetes, Hypertension, Dyslipidemia, Coronary Heart Disease, Gallbladder disease, Cancer and all-cause mortality.

Before going into further details into the pathology of obesity-related diseases that are primarily caused by excess adiposity in the body, it is important to first discuss what exactly is an adipose tissue and its main functions.

a. Functions of Adipose Tissue

Excess energy is stored in the lipid droplet of adipocytes which allowed the adipose tissues to be considered the main location of storage of body energy. When the individual is under a positive energy balance i.e. energy intake is higher than his body energy expenditure, this would result in either hyperplasia which is the increase in the number of the adipose cells or in hypertrophy which is a scenario of increase in the size of those adipocytes (Hausman *et al.*, 2001). The number of adipocytes that a person is holding in his body is mostly dictated during the early years of life going from childhood to teenage years. Following this period of life, weight gain mostly happens due to hypertrophy rather than hyperplasia; although few studies have proven that

hyperplasia can take place specifically more in the upper body adipose accumulation rather than lower body fat accumulation in adults with a normal BMI (Tchoukalova *et al*., 2010).

The adipose tissue can be divided into two categories: the white adipose tissue (WAT) and the brown adipose tissue (BAT). Both are found in adiposity areas (Medina-Gómez, 2012). Other than storing energy, WAT is believed to be the major endocrine organ in the human body (Kershaw & Flier, 2004). It has an endocrine function in addition to autocrine and paracrine roles (Kawai *et al*., 2012).

As for the BAT, it also has other utilities like dispelling energy in response to cold, hormones, thermogenesis after food intake, or sympathetic stimulation (Gil *et al*., 2011).

It is important to differentiate now between two types of white adipose tissues: SAT and VAT (Hayashi *et al*., 2008). VAT is known to proliferate at a slower rate and a lower capacity to differentiate thus it expands majorly by hypertrophy impairing the job of adipose cells. As for SAT, the expansion happens mainly via hyperplasia, until it reaches its limit thus hypertrophic alterations affects it. When both VAT and SAT are saturated, fat leaks to ectopic non-adipose locations (Tchernof & Depres, 2013).

Moreover, there is also likelihood for switching WAT into BAT and vice versa depending on its environmental exposure for instance, when being exposed to cold, some WAT becomes BAT for further provision of warmth. On the other hand, when being exposed to an obesogenic lifestyle, some BAT becomes WAT to allow for more storage of energy (Gesta, Tseng & Kahn, 2007).

Similarly to positive energy balance where the human body stores triglycerides in the adipocytes, negative energy balance, that usually occurs when a person is being physically active or fasting, has also its own repercussion. It puts the body in a state

where fat needs to be released from the adipose tissues under the action of specific enzymes like hormone-sensitive lipase (HSL), breaking the triglycerides (TGs) into its constituting elements and releasing free-fatty acids (FFAs) to the bloodstream to be received by peripheral tissues for energy. This process is referred to as lipolysis and is normally happening unless the amount of FFAs in the circulation is elevated. This would lead to obesity-related metabolic problems, including insulin resistance (Boden, 1997).

Many diseases developed due to the obesity scenario. The following section will give more background information about types of obesity, obesity-related diseases etiology & side effects and related-research & studies.

b. Patterns of Body Fat Distribution and Disease Risk

Recent studies have shown that different kinds of obesity have different side effects on the individual's health and the severity of the obesity-related diseases developed depends on the location of fat specifically rather than the degree of adiposity in absolute value.

The location of adipose tissue, viscerally specifically, is one major factor leading to all the health damages related to obesity. Several studies have supported the hypothesis of the pattern of fat distribution, not only absolute body fat percentage on its own. According to Larsson *et al*., statistically significant associations were found between the waist to hip circumference ratio and stroke occurrence ($p=0.002$) in addition to ischemic heart disease ($p=0.04$) (1984). Thus, this study confirms that the distribution of fat is a better predictor of metabolic health and cardiovascular diseases rather than the degree of adiposity.

Another prospective study conducted in Sweden on the risk factors for

ischemic heart disease followed the similar hypothesis: the waist-to-hip circumference ratio tested as a predictor for the development of diabetes mellitus. It has been conducted on 792 54-year-old participants for thirteen and a half years. Its results support previous findings of cross-sectional studies because the waist-to-hip circumference ratio showed positive and significant association with the development of diabetes mellitus even after taking BMI as a confounder (Ohlson *et al*., 1985).

After reviewing the different kinds of obesity and how different adiposity locations have different degrees in the severity of the side effects, it is crucial to give a summary on what those obesity-related diseases comprise.

c. Insulin Resistance and Hyperinsulinemia

Triglycerides stores in the adipose cells are promoted by the action of insulin via several pathways:

– It promotes the development of pre-adipocytes into mature adipose cells.

– In the mature adipocytes, insulin increases lipogenesis and the transport of glucose while hindering the levels of lipolysis.

– By influencing the phosphorylation status and function of proteins, in addition to affecting gene expression, insulin stimulates the activity of lipoprotein lipase (LPL) on circulating lipoproteins thus improves the uptake of fatty acids (FAs) into the adipose cells (Paradis & Ruvkun, 1998).

- *Insulin Resistance:*

Although it can happen due to genetic defects, obesity is an independent risk factor for a decrease in insulin sensitivity. A study conducted on twenty-three monozygous twins that differed in adiposity levels at around eighteen kilograms (Ronnemaa *et al*., 1997). This study concluded that only the individuals who had

majorly higher levels of visceral adiposity levels showed significant metabolic disturbances thus lower insulin sensitivity and tolerance to glucose following the ingestion of 75-g oral glucose tolerance test (OGTT). They also presented higher fasting insulin levels. The consequences of obesity don't refrain at affecting insulin levels but continue to reach metabolic alterations leading to hypertension, diabetes, dyslipidemia, higher blood coagubility, and cardiovascular diseases (Pi-Sunyer, 2002). This defect in insulin signaling is explainable at the cellular level. Malfunctional adipocytes subjected to hypertrophy mainly found in VAT and SAT of the upper compartment of the body result into a high release of FFAs due to their lipolytic feature, in addition to the release of adipokines like higher leptin and lower adiponectin into the circulation, act as main contributors in the development of obesity-induced insulin resistance (Castro *et al*., 2014).

Two major assumptions justify the association between the elevation of FFAs and insulin resistance: The portal and the spillover hypotheses (Castro *et al*., 2014).

Starting with the portal hypothesis, it is thought that due to the presence of abdominal obesity and increased release of FFA towards the liver which would be the preferred substrate for energy production, insulin resistance takes place hepatically. This would lead eventually to uncontrolled hepatic glucose synthesis and release into the bloodstream. The impaired glucose utilization by the liver leads to higher glucose levels in the circulation known as hyperglycemia. Adding it to the higher hepatic glucose production, and ultimately impaired glucose tolerance, diabetes mellitus tends to develop (Jensen *et al*., 1989). Higher FFA turnover rate, lower glucose disposal rate (GDR) and higher liver production of glucose was shown in females who present abdominal adiposity when compared to ones with peripheral adiposity (Jensen *et al*., 1989; Peiris *et al*., 1988).

In addition, it has been added that as well as the release of FFA affects insulin resistance, the release of inflammatory adipokines from VAT play an equal role (Konrad, 2012).

As for the spillover theory, it is believed that after the adipocytes reach their maximum limit of expansion, FFAs leak to non-adipose tissues as well as to VAT areas. Yet remains the problem of the restricted capacity of those non-adipose tissues to handle FFA and its byproducts, whether in oxidation or in storage, which why insulin resistance develops besides cell apoptosis and hindered related organ function (Virtue & Vidal-Puig, 2010). Moreover, fat accumulation intracellularly in non-adipose tissues stimulates the production of toxic derivatives via secondary reactions, which leads to an impaired function known as lipotoxicity and consecutive cell death renowned as lipoapoptosis (Slawik & Vidal-Puig, 2006).

Over and above, the latter area suffers from hypoxia and subsequently endoplasmic reticulum (ER) stress due to oversized adipocytes. ER stress occurs when the ER is unable to perform protein folding properly because of excess fat and glucose accumulation in addition to hypoxia. The buildup of proteins that are not well folded or unfolded produces ER stress. The intention of ER stress is to stop the translation of misfolded proteins while stimulating other processes for assistance. When the latter fails, reactive oxygen species (ROS) build up, inflammatory reactions and cell dysfunction and death take place (Ye, 2013). Cell death and permeation of macrophages entry which lastly result in higher release of inflammatory cytokines like TNF-α, IL-6 and monocytes chemoattractant protein-1 (MCP-1) producing a status of low-grade inflammation locally & systemically and further exacerbation of the impairment in insulin signaling (Tchernof & Després, 2013).

- *Hyperinsulinemia*

Pancreatic insulin production and hepatic insulin clearance are the main actors in producing insulin balance physiologically (Ader *et al*., 2014). Insulin gets cleared by taking it into the tissues or by degrading it. When it comes to the liver, insulin gets cleared via receptor mediation. Many factors might affect insulin clearance and uptake, like FFA levels (Stears *et al*., 2012).

In cases of obesity, high levels of glucose and FFA in the blood stimulate higher pancreatic insulin production and the developing IR leads to lower hepatic clearance which would ultimately promote hyperinsulinemia. The latter lowers the clearance of insulin from the bloodstream by down-regulating insulin receptors (Stears *et al*., 2012).

d. Type II Diabetes

As BMI increases, the chance to develop type II diabetes mellitus (T2DM) increases. This statement has been proven by several studies on both genders. In a study conducted on females, as her BMI increases from 22kg/m^2 to 35kg/m^2, the risk to develop diabetes increases 93 times (Chan *et al*., 1994). The scenario is not much different with males. BMI alone as well as weight gain alone independently of the BMI were independent risk factors to grow T2DM in 51,000 male health professionals aging 40 to 75 years. At their 21st year of life, males with a BMI higher than 24kg/m^2 or who gained weight of more than 11kgs were at a 21 times higher risk to develop T2DM compared with males who gained less than 5kgs of weight or with a BMI lower than 22kg/m^2 (Pi-Sunyer, 2002). Furthermore, in a cohort study conducted for 13 and a half years on 1913th-born men, the lowest tertile of waist-to-hip ratio had a 30 times lower risk to develop diabetes compared to the ones with the highest tertile whereas men in

the highest BMI tertile yet with low waist-to-hip ratio did not present any increased risk of T2DM (Ohlson *et al*., 1985).

e. Inflammatory Status & its Relation to Insulin Resistance

Obese people are known to have higher levels of adiposity due to the excess fat that they accumulated in their body throughout their life. The adipose tissue shares an endocrine function in addition to its function of storing fat inside their cells. The inflammatory markers released by adipocytes are known as proinflammatory cytokines, or adipokines (del Giudice *et al*., 2009). This puts obese individuals, having excess adiposity, in a state of chronic low-grade inflammation.

Inflammation and insulin resistance: Triggered by nutritional stress & detected by immune sensors

Cytokines that Link Inflammation to Insulin Resistance:

- *Tumor Necrosis Factor-α (TNF- α):*

It is a pro-inflammatory cytokine released from adipose cells that might induce the occurrence of insulin resistance by stimulating lipolysis in adipocytes and by enhancing the phosphorylation of serine/threonine in IRS-1 (Shoelson, Lee, & Goldfine, 2006).

After the stimulation of the adenosine monophosphate activated protein kinase (AMPK) by TNF-*α*, glucose uptake is higher in both subcutaneous and visceral adipose cells. Yet, in VAT specifically, it promotes insulin resistance by stimulating JNK1/2.

In studies trying to find therapies to insulin resistance, soluble tumor necrosis factor-like weak inducer of apoptosis (sTWEAK) were used in studies, to obstruct TNF signaling which proves the role the latter has in this mechanism (Vázquez-Carballo *et al*., 2013).

- *Interleukin-1β (IL-1β):*

IL-1β is a pro-inflammatory cytokine which also aids in promoting insulin resistance yet by a different mechanism than TNF-α. IL-1β disrupts the insulin signaling in both macrophages and peripheral cells which as well includes pancreatic β cells. Therefore, this would decrease the sensitivity of the latter to insulin thus altering their secretion of insulin (Böni-Schnetzler & Donath, 2013).

The secretion of IL-1β is dictated by the activity of the inflammasome and in different body tissues like monocytes, IL-1β level is elevated when the body is under a hyperglycemic state (Koenen *et al*., 2011).

In type-II diabetes mellitus (TIIDM), a major contribution is awarded to IL-1β in beginning and sustaining organ dysfunction that is provoked by inflammation (Grant & Dixit, 2013).

In the main insulin-acting sites like macrophages that are considered as a target location for insulin activity, IL-1β is able to restrain insulin activity as well as its capacity of further exacerbating and advancing the human body systemic inflammatory status (Hardaway & Podgorski, 2013).

- *Interleukin-6 (IL-6):*

IL-6 has no less of a contribution in the development of insulin resistance. It is released by several cells, and majorly adipocytes. It also intensifies IR by down-regulating the expression of IRS-1 & glucose transporter-4 (GLUT-4). Furthermore, IR can be exacerbated by blocking the PI3K pathway as well as hindering glycogen synthesis. Some studies show that IL-6 is responsible for IR at the level of the skeletal muscle which happens by activating STAT3 pathway and ultimately stimulating the Toll-Like Receptor-4 (TLR-4) genetic expression (Kim *et al*., 2013).

- *Leptin:*

Leptin resistance is observed in obese individuals. Leptin, being a protein released by WAT, has anti-inflammatory properties, reduces appetite and stimulates energy expenditure in the human body by affecting neuronal circuit in the hypothalamus: activating catabolic ones and inhibiting anabolic ones. In addition, leptin is being linked to IR since overweight and obese people suffer from a state of leptin insufficiency in the hypothalamus and leptin is as well crucial for glucose metabolism because it is involved in the regulation of PI3K pathway and in the function of the pancreatic β-cells (Hardaway & Podgorski, 2013). The latter is why this anti-inflammatory leptin has been considered as a potential therapeutic approach to improve insulin sensitivity, glucose and lipid metabolism (Yau *et al*., 2014).

- *Adiponectin:*

Adiponectin is a cytokine secreted by the WAT, has two different receptors of different functions and is involved differently in different diseases. In cases of obesity, diabetes type II, or insulin resistance, levels of adiponectin decrease due to its anti-inflammatory functions whereas in scenarios of osteoarthritis, or diabetes type I, levels of adiponectin witness an increase, acting as a pro-inflammatory cytokine (Passos & Gonçalves, 2014). Adiponectin is linked to improving IR due to two receptors that link it to glucose metabolism. The first one is Adiponectin Receptor 1 (AdipoR1) is highly released in the skeletal muscle, acts by activating AMPK thus reducing the expression of molecules involved in lipogenesis in addition to decreasing the expression of genes encoding for hepatic gluconeogenic enzymes. As for Adiponectin Receptor 2 (AdipoR2), highly produced in the liver, it improves glucose consumption by stimulating PPAR-α signaling (Crimmins & Martin, 2007; Yamauchi & Maki, 2007).

- *Resistin:*

As opposed to the resistin in rodents highly secreted by adipocytes, macrophages are the main location for its synthesis in humans. Whenever the levels of inflammatory facilitators increase, resistin levels act similarly. It has been thought to be pro-inflammatory, worsening IR and found in high levels mainly in obese and overweight individuals (Szulinska *et al*., 2014). Resistin exacerbates the situation of IR by stimulating the expression of pro-inflammatory cytokines like IL-6 and TNF-α. Moreover, it is involved as well in the worsening of IR and exacerbating the individual's inflammatory status by activating the MAPK and JNK signaling pathways after binding to TLR-4 hypothalamic receptors (Benomar *et al*., 2013).

- *Monocyte Chemo-attractant Protein-1(MCP-1):*

Monocyte chemoattractant protein-1 (MCP-1) is as well added to the list of pro-inflammatory chemokine which is released by macrophages, adipose cells and endothelial cells. MCP-1 recruits dendritic cells, memory T-cells and macrophages (Kanda *et al*., 2006). Because adipocytes and macrophages release MCP-1 in the highest amounts, levels of MCP-1 are elevated in cases of excess adiposity and obesity. The latter would lead to further recruitment of macrophages and DCs and ultimately the release of cytokines which will aggravate inflammation-caused IR (Kahn *et al*., 2006). Because MCP-1 is more released by VATs, it is also majorly involved in the development of IR, particularly hepatically (Kouyama *et al*., 2007). By affecting lipid metabolism, the infiltration of macrophages, the inflammatory status of an individual and insulin sensitivity, MCP-1 is acknowledged to be associated with a higher inflammatory profile and worsened IR (Nio *et al*., 2012).

- *Macrophages:*

When macrophages infiltrate to get inside the adipocytes, they will be labeled

as “Adipose Tissue Macrophages” or ATMs. ATMs act largely in the development of obesity-derived IR. ATMs are of two types: classically activated ATMs (M1) in animals with excess weight and adiposity, and alternatively activated ATMs (M2) in lean species (Fujisaka *et al*., 2009). Being a major site for the production of cytokines, macrophages acquire a huge intervention in the development of chronic inflammation which as well includes inflammation of obesity.

- *C-Reactive Protein (CRP)*

C-reactive protein (CRP) is a hepatically-synthesized acute-phase inflammatory marker whose levels are elevated in cases of inflammation. Pro-inflammatory cytokines like IL-6 and TNF-α control the level of CRP by affecting its regulation (Spruijt-Metz *et al*., 2012). It is acknowledged as high-sensitive CRP (hs-CRP) (Alemzadeh & Kichler, 2014). Usually, insulin suppresses the release of CRP to prevent its elevated levels and high CRP levels are caused by a low insulin suppression of CRP synthesis. In addition, vascular inflammation is also affected by CRP level by stimulating complement proteins and elevating the synthesis of thrombogenic compounds that usually bind to the membrane of injured vascular cells (Hanyu *et al*., 2009). It has been also believed that elevated CRP levels can be linked to higher risk of diabetes type II. However, no causality has been established between IR, diabetes and CRP levels. The latter shows that CRP levels are mere indicators of the pathway rather than elements involved in the pathogenic pathway (Brunner *et al*., 2008). Yet, CRP is highly linked to IR and CRP expression is always kept under sight when studying IR.

f. <u>Dyslipidemia</u>

An additional consequence of obesity occurrence is not only restricted to impaired glucose tolerance, diabetes or insulin resistance. In fact, the previously

discussed higher FFA turnover, in addition to producing and worsening the situation of insulin resistance, also increases the number of circulating very-low-density lipoproteins (VLDLs). This leads to the production of small-dense high-density lipoproteins (HDL) and small-dense low-density lipoproteins (LDL). The small-dense HDL are renally cleared thus the obese individual observes lower HDL levels. As for the LDL levels, although the levels may not change, yet the small size the LDL particles acquired allow them to slip into the arterial wall where they are not protected by antioxidants against free radicals. Ultimately, oxidation and glycation takes places which make them more atherogenic than the large LDL particles which are big enough to prevent slipping into the subendothelial space (Despres, 1994).

g. Coronary Heart Disease (CHD)

The risk of mortality from CHD was found to be associated with fasting insulin levels independently (Pi-Sunyer, 2002). In a Canadian case-control study conducted on men, even after adjusting for LDL, HDL, plasma triglycerides and apolipoprotein B, a significant association was still found between CHD and insulin levels (Despres *et al*., 1996).

Furthermore, CHD risk was also associated with the individuals' BMI levels. Non-smoker obese individuals in the Nurses' Health Study had increased CHD risk 5.8 times when their BMI reached 32kg/m^2 compared to the ones with a BMI of 22kg/m^2 (Manson *et al*.,1995). More specifically, the higher the quintile of waist-to-hip ratio, the risk of CHD was higher of 8 times compared to a lower waist-to-hip ratio quintile.

h. Gallbladder Disease

In the Atherosclerosis Risk in Communities Study, hospital admissions for

gallbladder diseases in 12,700 individuals aging between 45 and 64 years of age was higher by 45% in overweight females compared to lean ones (Boland *et al*., 2002). In addition, the waist-to-hip ratio doubled the risk of gallbladder diseases in women compared to the lowest quartile; whereas, the waist-to-hip ratio showed no changes in males individuals.

i. Social Burden

The first consequence to mention is the social burden that a person's obesity status puts on his life. According to Puhl *et al*., obese and overweight individuals are discriminated (2001). They are more likely to be less paid compared to normal-weight individuals performing the same job, more likely to be subject to inferences of laziness by their bosses at workplaces as well as they have lower employment opportunities. With the same qualifications, it has been shown that an employer is more likely to choose the thin candidate over the overweight or obese candidate to take the job. Moreover, healthcare professionals tend to have more negative attitudes towards obese and overweight individuals. This has been proven to be one of the reasons why overweight individuals tend to be more reluctant in seeking help and healthcare (Puhl *et al*., 2001).

j. Depression

An additional consequence of obesity to the above is the psychological disturbances among many. According to Luppino *et al*., overweight and obesity, separately, increased the risk of onset of depression among adults aging between 20 & 59 years and even among elderly, aging more than 60 years of age (2010). Studies have shown that due to the discrimination that obese people are subject to, they were found to

have a double risk of being depressed at baseline and after follow-up measurements (Roberts *et al*., 2003). In addition, the effect is reciprocal. Further studies have been focusing on the causal effect that depression might have on obesity. The rationale behind this type of studies was based on behaviors like emotional eating and depression-induced low physical activity levels (Dallman *et al*., 2005). A meta-analysis combining studies that involved over 33,000 subjects supported the hypothesis that depressed non-obese individuals tend to binge-eat, especially on high-caloric food compared to non-depressed individuals. This was even more reinforced in depressed non-obese dieters compared to non-obese non-dieters (Blaine, 2008).

The health consequences of obesity are numerous and can have detrimental effects on the human health, daily life and well-being. However, obesity does not impact the human body separately from iron. On a long run, due to the low inflammatory status caused by obesity, iron deficiency develops and if persisting, it will develop into a specific type of iron-deficiency anemia known as "Anemia of Chronic Disease."

5. Anemia of Chronic Disease

Anemia of chronic disease (ACD) which is also called anemia of inflammation takes place when a chronic disease causes chronic inflammation leading to iron deficiency and ultimately IDA. ACD is considered to be the highest in hospitalized patients (Weiss & Goodnough, 2005). ACD can affect 8-95% of patients that are either infected or have malignancies or suffer from auto-immune disorders (Nissenson *et al*., 2003). Age, cancer and therapeutic measures including radiotherapy or chemotherapy aggravate the onset and worsening of ACD in those patients (Dunn *et al*., 2003). After going through the pathophysiology of ACD, three factors have been thought to be the

reason behind it.

First of all, during inflammation, iron will be retained inside the cells of the RES which leaves little iron available for erythroid progenitor cells which on the longer run leads to the occurrence of anemia.

In addition to that limited iron availability for erythroid progenitor cells, the multiplication and differentiation of those cells is also negatively affected due to the presence of cytokines and acute-phase proteins. α-1 antitrypsin and α-2 macroglobulin bind to transferrin receptors (tfR) which would prevent erythroid progenitor cells from uptaking iron via those tfR which would ultimately block their proliferation (Weiss, 2009).

The third important factor by which inflammation affects ACD is by affecting the endogenous erythropoietin which is the hormone that mediates the conversion of the erythroid progenitor cells to erythrocytes. Whether by reducing its formation or by decreasing its biological activity, ACD leads to inadequate erythropoietin levels. This is manifested, for instance, by an impaired biological response of the individual to hypoxia (Theurl *et al*., 2006).

a. Hepcidin: A Key Regulator in Iron Metabolism

Being a renowned small antimicrobial peptide produced by the liver, studies have proven that hepcidin has one of the most important roles as a hormone involved in iron metabolism (del Giudice *et al*., 2009). The liver originally produces a pre-peptide that is processed proteolytically to obtain a mature hormone ready to function. The latter is an eight-cysteine residues-containing protein comprising 25 amino acids, encoded by a gene known as HAMP.

Although it is mainly produced hepatically, it is mildly produced in small doses

by extra-hepatic tissues like heart, brain, pancreas etc, in addition to macrophages. However, it came to a realization that only liver-produced hepcidin is involved in iron metabolism and homeostasis.

Two factors appear to control hepcidin transcription and blood levels:

- High iron levels in the body stimulate an increase in hepcidin levels to prevent elevated intestinal iron absorption of consumed dietary iron.
- Inflammation induced hepcidin release aiming to cause low iron blood levels (hypoferremia) by restraining iron inside the macrophages. This is recognized as 'nutritional immunity' with the intention to deprive bacteria from iron considered as an essential nutrient for its growth and proliferation. Being also an antimicrobial, this as well adds up to the role hepcidin plays in defending hosts from any external microbial invasion. This shows how hepcidin acts as a role between native immunity and iron homeostasis.

On the contrary, other situations like low iron levels or anemia and cases of hypoxia lead to decreased hepcidin levels by inhibiting its expression. This would lead to an increase in ferroportin thus iron flux to the bloodstream to adequately meet body iron needs for erythropoeisis (Papanikolaou & Pantopoulos, 2017).

Hepcidin regulates iron absorption through different pathways:

- Hepcidin regulates the intestinal absorption of iron when it binds to ferroportin in cells mainly intestinal cells and macrophages. Consecutively, ferroportin gets internalized and degraded.
- Hepcidin also controls iron release from macrophages which would as well affect the amount of iron that is readily available for erythropoeisis. Studies on mice show that the ones with an inactive hepcidin gene observed iron overload compared to other mice with an overly expressed hepcidin gene that showed an opposite scenario of

severe anemia of iron deficiency (Andrews, 2008).

Iron-refractory iron deficiency anemia (IRIDA) has been observed in scenarios where the gene TMPRSS6 encoding for matriptase-2 is inactivated or mutated. It is important to mention that matriptase-2 is a negative regulator for hepcidin which acts by inhibiting the action of hemojuvelin (HJV) by cleaving it. HJV is a liver-secreted protein capable of regulating hepcidin levels, yet its exact function is still unclear. Matriptase-2 is used as the escort when iron levels are low. When IRIDA develops, hypochromic anemia is observed which cannot be treated with iron supplements orally. Under situations where inflammation is chronic, hepcidin levels remain high and macrophages keep hold of iron inside them as a defense mechanism. Subsequently erythropoeisis is affected and ultimately anemia of chronic disease (ACD) develops. Other co-factors that speed up the development of ACD exist like induced ferritin transcription and decreased ferroportin levels however, studies show that hepcidin treatment solely is a therapeutic option.

As hepcidin regulates iron absorption and blood iron levels, the latter also have an effect on hepcidin transcription and release at the level of the liver. Although unclear, it is believed that the iron levels affect the promoter of hepcidin through BMP/SMADs. When liver iron levels are high, BMP6, among other BMPs, is released. BMP6 binds to HJV & BMP receptors. BMP6 will phosphorylate SMAD and translocate it from the cytosol of the hepatic cell to its nucleus to activate the promoter of the HAMP gene encoding for hepcidin. Thus, iron levels will be decreased and brought back to normal (Figure 1).

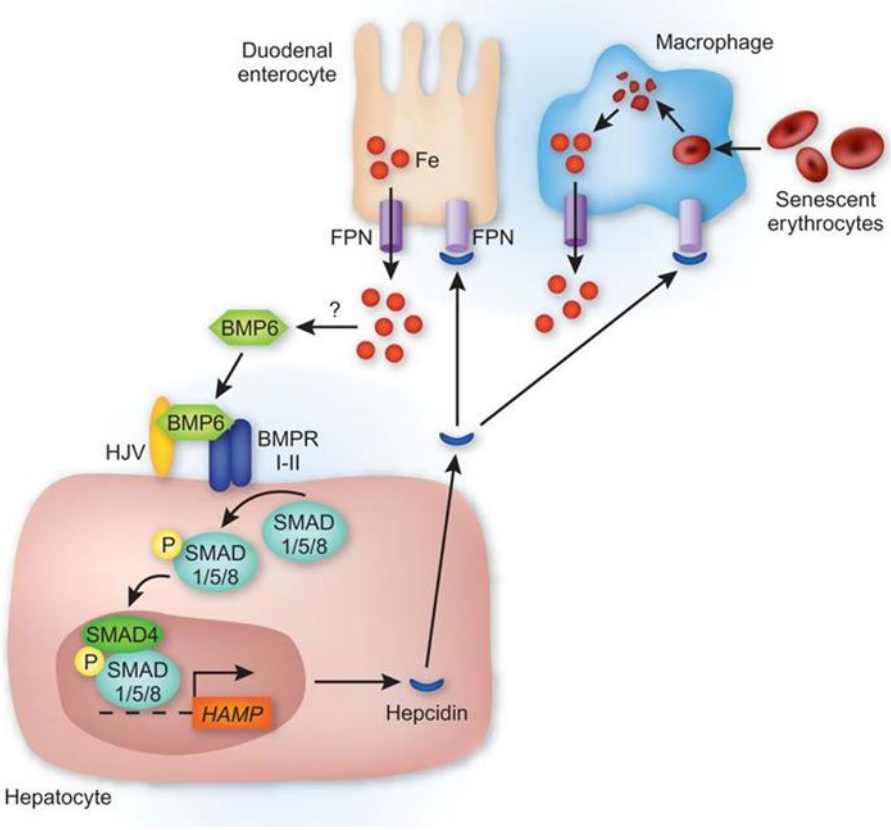

Fig. 1. Hepcidin Regulation
Source: Gupta *et al*., 2012

On the other hand, when iron levels are low, BMP-6 is also responsible of inhibiting the action of hepcidin by up-regulating the expression of matriptase-2.

b. Hepcidin and Inflammation

As previously mentioned, in overweight or obese people, a high inflammatory status leads to a condition known as anemia of chronic disease (Andrews, 2008). In order to verify the link between obesity and inflammation, studies have shown that hepcidin is not only released by the liver, but also expressed by adipose tissue. In fact, the mRNA expression of the hepcidin gene increases when the amount of adipose tissue increases in obese people.

According to Andrews (2004), experiments have been conducted in order to link inflammatory markers with hepcidin levels. They have worked on both mice and human models. They showed that inflammatory cytokines had no effect on increasing hepcidin production levels. On the other hand, TNF-α decreased it; however, IL-6 in vivo induced hepcidin increased that was followed by hypoferremia. The same has been studied on mice where inflammatory abscesses have been induced in both the wild-type and IL-6 knockout mice. The wild-type mice showed a similar increase in hepcidin levels leading to lower serum iron levels as observed before but the IL-6 knockout mice did neither increase hepcidin nor decreased serum iron levels. Similar experiments have been conducted on human participants where an infusion of IL-6 resulted on increased urinary hepcidin excretion that is further leading to hypoferremia. That acts as strong evidence that IL-6 is a primary factor that stimulates hepcidin levels production and excretion while lowering serum iron levels.

In spite of this correlation, studies showed that high hepcidin levels are insufficient factor to diagnose a patient with anemia of inflammation. This concept took place due to the analysis of mice with thalassemia. Those mice are presumably with high serum iron levels yet they do not fail to show low hepcidin levels.

As a small conclusion of all the previous data analyzed, IL-6 stimulates hepcidin release from hepatocytes which will decrease iron absorption at the level of the intestines and iron release from macrophages.

At the cellular level, IL-6 promotes hepcidin release through the JAK/STAT pathway. IL-6 will attach to receptor complexes identified as gp130. Successively, STAT3, the transcription factor, will be phosphorylated with the mediation of JAK 1/2. At the HAMP gene promoter, STAT3 will bind to STAT3-binding site (STAT3-BS) thus hepcidin transcription is stimulated.

c. Hepcidin Assessment and Possible Therapeutical Approach

It is important to mention here that for accurate measurements of hepcidin levels; it is better sampled from urine rather than serum because hepcidin gene expression is regulated while hepcidin is a small peptide that is hardly filtered by nephrocytes.

Many have suggested that the easiest treatment to this scenario is blocking the increase in hepcidin levels which would prevent hypoferremia.

However, this is more easily said than done because if healthcare professionals tend to consider this as a treatment, even if it would work in patients with non-infectious inflammatory disorders, it would be harmful for people who present infections or malignancies.

This is because the increase in hepcidin levels and the decrease in serum iron levels act as a defense in the host against pathogens and tumor cells. If hepcidin was blocked and serum iron levels remain relatively high in the serum, this would prevent the antimicrobial defensive properties of hepcidin to be exerted.

The previous studies mentioned before show that the amount of adiposity a person has is capable of affecting his visceral fat levels thus worsening his metabolic health by exacerbating his inflammatory level, insulin level and subsequently his absorption of the mineral of interest in this study: Iron. The study conducted in this thesis focuses on what the world of research lacks, which is the effect of fat location specifically rather than absolute adiposity on the predictors of metabolic health: inflammation and insulin resistance, and ultimately iron absorption.

CHAPTER III

MATERIALS AND METHODS

This chapter illustrates the study design. It provides details about the sample size, sample baseline characteristics, the chosen and applied sampling method. Laboratory work and samples analysis are as well portrayed. Moreover, this section contains a special section for the methods used in our statistical analysis and the definition of our statistical variables.

A. Study Population

In general, everyone is subject to iron deficiency and iron deficiency anemia if untreated. However, specific subgroups of the population are of higher risk: children below 5 years of age, women of childbearing age and pregnant females. This thesis focuses on one group out of those three, which is a woman of childbearing age. The latter is the main reason why our sample is solely composed of pre-menopausal females aging between 20 and 50 years of age. They are chosen to fit into one of three categories: females of normal weight, overweight females and obese females. The sample taken is from the Lebanese population where the females come from different Lebanese areas. The demographic distribution of our sample doesn't affect the hypothesis since it is a physiological mechanism that is not affected by the socio-economic status of the individuals. Specific inclusion and exclusion criteria, to be mentioned in following segments, are applied to eliminate possible confounders from affecting the accuracy and credibility of the obtained results.

B. Sampling Selection

In order to recruit participants for this study, random sampling selection was performed. Every individual of the population that fits the inclusion criteria of the study had an equal chance of participating in the study. However, during later stages of recruitment, people used to refer their acquaintances to participate in the study which made the sampling selection become a snowballing selection or referral. Due to the fact that the researchers are university students whose social surrounding wasn't a helping factor to find people from different age categories where inclusion criteria applied, snowballing made this problem solved. In addition, younger participants got recruited with the word of a mouth hearing about the study being conducted at the American University of Beirut and they approached the researchers to volunteer in the study.

C. Procedure

The first step of the study is the recruitment phase. Potential participants were contacted by phone, the study was explained to them, and interested ones will be checked if they fit the inclusion criteria of the study. Females aging between 20 and 50 years, with a BMI above 18.5kg/m2 were included in the study if they are: Non-pregnant, non-lactating, or have not undergone any surgery or donated blood recently. On the other side, females who are either pregnant or lactating will be excluded, due to their hormonal instability that may affect blood glucose and iron absorption rates, in addition to the altered adiposity level and distribution. Individuals suffering from diseases that may be affecting their blood, such as thalassemia, or subjects with Hb<11g/dl, or any other type of nutritional or non-nutritional anemia, or with any inflammatory diseases, known diabetes or dyslipidemia, or suffer from GI conditions that affect iron absorption, or take medications that affect carbohydrate metabolism or

on proton pump inhibitors (PPIs) shall as well be excluded from the sample population.

After checking for inclusion/exclusion criteria, matching participants will be invited for screening at the American University of Beirut Medical Center (AUBMC).

1. Screening Day

Potential participants are asked to come to AUBMC. They will be asked to thoroughly read the consent form and sign it. They will be asked to fill a questionnaire about their socio-economic, demographic and health status. Following it, anthropometric measurements, including weight (kg), height (cm), waist circumference (cm), BMI (kg/m2) will be taken for consented individuals then their body composition will be scanned by a trained professional using the Discovery Dual Energy X-Ray Absorptiometry (DXA) Hologic Horizon A Apex version 13.6.05.

According to the results of the DXA machine, participants will be classified as lean, overweight or obese (centrally or peripherally). An appointment will be scheduled for the first experimental day.

2. Experimental Day #1

On the first experimental day, participants are asked to attend at the experimental room following an overnight fast. They are also asked to avoid caffeinated beverages, smoking and any strenuous physical activity one day prior to the test and on the day of the test itself. A blood sample will be collected from the participants to measure their complete blood count (CBC), C-reactive protein (CRP), α-glycoproprotein, hepcidin, serum ferritin, transferrin receptors (TfR), saturated transferrin %, serum iron, Total iron binding capacity (TIBC), HbA1C, fasting plasma insulin, fasting blood glucose (FBG), and lipid profile.

After the blood withdrawal, a test meal composed of 80-90g of bread with 10g of butter and 30g of honey, 6 mg of Fe^{57} which are aliquoted and imported from ETH Zürich university in Switzerland, will be given to the fasting participants. 300ml of deionized water, collected from de-ionized water dispenser at the American University of Beirut, is also given with the test meal. Participants will be asked not to eat or drink for 3 hours after the test meal to avoid any impact of consumed foods on the absorption and digestion of the ingredients of the test meal, specifically iron.

3. Experimental Day #14

After 14 days since the test meal day, participants are asked to attend to the experimental room once again. After an overnight fast, baseline blood sample will be collected. Afterwards, they will be asked to have the oral glucose tolerance test (OGTT) drink which contains 50 grams of glucose, imported from the American biotechnology company Azer Scientific in Morgantown, and 100mg of Fe added to it in the form of 480mg of sodium ferrous citrate or Sanferol which is obtained from a Japanese pharmaceutical company.

After waiting for 2 hours with minimal movement, participants' blood will be collected as the final blood sample to be analyzed. All blood samples were frozen at -80°C. They will be used for analysis of biochemical markers.

Glucose, lipid profile, serum Fe, Total Iron Binding Capacity (TIBC) and transferrin saturation are measured using VITROS® 250/350 Chemistry System. As for insulin and hepcidin, they are measured by the enzyme-linked immunosorbent assay (ELISA) using Thermo Scientific Multiskan GO version 1.00.40. Complete Blood Count (CBC) is performed by the American University of Beirut Medical Center (AUBMC). Moreover, HBA1C is computed using the DCA Vantage® Analyzer in

American University of Beirut. As for inflammatory markers (C-reactive protein (CRP), α-glycoproprotein & serum ferritin), transferrin receptors (TfR) & erythrocyte iron enrichment, they are assessed in collaboration with a team of professionals from the laboratory of human nutrition in ETH Zürich University in Zürich, Switzerland.

When results are obtained, in case of any abnormal figures, participants are requested to consult with their physicians for appropriate treatment and the physicians' approval whether to include or eliminate the results from the data pool.

CHAPTER IV

RESULTS

A. Subjects' Characteristics

This study recruited 121 participants after being tested for inclusion criteria. Three participants dropped out of the study for personal reasons unrelated to study activities. The remaining 118 recruited participants participated in this study. Our sample of study was divided into 4 groups of different BMI: Lean group (18.5≤BMI<25), Overweight group (25≤BMI<30), Obese class I (30≤BMI<35), Obese class II (BMI≥35). The subjects' characteristics are summarized in Table 1.

Table 1. Subjects Characteristics

	Lean (n=37)	**Overweight (n=27)**	**Obese Class I (n=28)**	**Obese Class II (n=26)**	**ANOVA *p*-value**
Age (y)	31.68±9.42[a]	32.0±9.38[ab]	35.21±9.61[ab]	37.65±7.54[b]	0.043*
Height (m)	1.62±0.05	1.61±0.05	1.60±0.07	1.60±0.05	0.203
Weight (kg)	59.95±5.67[a]	70.70±7.00[b]	82.50±8.14[c]	98.90±11.98[d]	0.0005*
WC (cm)	75.43±7.31[a]	86.56±8.37[b]	96.18±7.21[c]	109.40±8.63[d]	0.0005*
BMI (Kg/m^2)	22.75±1.63[a]	27.25±1.61[b]	32.36±1.25[c]	38.64±3.25[d]	0.000*

* Statistically significant (*p*-value<0.05)

* Groups having different subscripts are statistically different according to Bonferroni post-hoc analysis

Mean age has been increasing from around 32 years in lean group to around 38 years in obese class II. The difference in means of age among the 4 groups was statistically different but according to Bonferroni post-hoc analysis, it is only significant difference in age was between normal BMI group and obese class II group (*p*-value=0.035<0.05).

Weight has increased from around 60kgs in lean group to around 99kgs in obese class II group. Between the first three groups, the increase was on average by 10kgs. Yet, it jumped to 20kgs approximate difference between obese class I and obese class II.

Waist circumference ranged between 75.4cm to 109.4cm comparing means of 4 BMI groups. It increased between the 4 BMI groups by 10cm on average. This shows that even when weight difference jumped to 20kgs between the last 2 BMI groups, waist circumference hasn't been proportionally increasing.

BMI followed the same path with an increase from 22.8kg/m^2 in lean group to 38.64kg/m^2 in obese class II. The average increase between each two consecutive BMI groups is 5kg/m^2.

As for height, we can see that the mean difference is not statistically significant.

B. Body Composition

Mean estimated visceral fat area has increased from 62cm^2 to 182cm^2 going from lean group to obese class II with an average increase between the 4 BMI groups around 40cm^2.

As for mean gynoid fat, it also increased going across the BMI lane from around 5kgs in lean group to around 9kgs in obese class II group with an average increase of 1kg among the 4 BMI groups.

Furthermore, android fat increased from 1.44kgs in normal-weighted individuals going to around 4kgs in obese type II individuals with an average increase between the 4 BMI groups was around 1kg.

Table 2. DEXA Analysis according to One-way ANOVA and Bonferroni post-hoc analysis

BMI Group	Lean (n=37)	Overweight (n=27)	Obese Class I (n=28)	Obese Class II (n=26)	One-way ANOVA *p*-value
Android Fat mass (Kg)	1.44±0.47[a]	2.26±0.38[b]	3.18±0.52[c]	4.13±0.82[d]	0.000*
Android Fat (%)	35.36±6.05[a]	43.97±3.18[b]	48.92±3.23[c]	50.99±4.24[c]	0.000*
Gynoid Fat Mass (Kg)	4.79±0.97[a]	6.28±1.09[b]	7.24±1.18[c]	8.56±1.74[d]	0.000*
Gynoid Fat (%)	44.11±4.17[a]	48.04±3.29[b]	49.14±3.35[b]	49.96±4.25[b]	0.000*
A/G Ratio	0.80±0.09[a]	0.92±0.07[b]	0.99±0.05[c]	1.02±0.08[c]	0.000*
Percent Body Fat (%)	38.09±4.76[a]	44.15±2.72[b]	46.87±2.66[c]	49.14±3.45[abc]	0.000*
Total Fat Mass (Kg)	23.89±4.59[a]	32.54±4.24[b]	40.17±4.99[c]	50.47±8.39[d]	0.000*
Est. VAT Area (cm^2)	63±19[a]	94.78±30.18[b]	148.30±39.34[c]	181.58±42.02[d]	0.000*
Lean Body Mass (Kg)	38.44±3.24[a]	41.06±3.99[a]	45.41±4.33[c]	51.91±5.80[d]	0.002*
Lean Body Mass (%)	61.92±4.76[a]	55.87±2.72[b]	53.13±2.66[c]	50.87±3.45[c]	0.004*

* Statistically significant (p-value<0.05)

* Groups having different subscripts are statistically different according to Bonferroni post-hoc analysis

Percent body fat showed similar results with an increase from 38% mean percent body fat in lean group to 49% in obese class II with an average increase of 5% body fat between the first three groups yet reaching the last BMI group, increase in percent body fat dropped to only 2%.

As for A/G ratio, table 2 shows that it was statistically significant among all BMI groups moving from normal to overweight to obese class I. As we go from obese class I to obese class II, the A/G ratio was not statistically significant anymore which shows that the increase in adiposity in those classes leads to a proportional increase between android and gynoid areas.

Although among the last 2 obese classes, A/G ratio wasn't statistically

different, visceral fat area remained statistically significant among the 4 BMI classes. This means that even if the increase in adiposity was proportional among android and gynoid areas, it still had a significant effect on visceral fat areas.

The last body composition marker is lean body mass where an opposite scenario has been observed. Mean lean body mass was the lowest in lean group reaching 38kgs increasing to around 52kgs in obese class II group. Significant difference was seen in percent lean body mass between all groups where the only insignificance was between lean and overweight groups. As for the lean body mass percent, the figure decreased going from 62% in lean group to 51% in obese class II group where all groups were significantly different except for insignificance between obese class I and II groups.

C. Hematological Analysis

None of the hematological markers was statistically significant among 4 BMI different groups.

Table 3. Hematology Analysis according to One-way ANOVA and Bonferroni post-hoc analysis

BMI Group	Lean (n=37)	Overweight (n=27)	Obese Class I (n=28)	Obese Class II (n=26)	ANOVA *p*-value
Hemoglobin(g/dl)	12.91±1.08	12.83±0.94	13.14±1.17	12.54±1.66	0.346
n (%) of Hb<11g/dl	*5 (13.5)*	*2 (7.4)*	*0 (0)*	*4 (15.4)*	
RBC(mil/mm3)	4.71±0.33	4.69±0.47	4.59±0.34	4.65±0.32	0.568
MCV (fL)	81.21±7.02	82.70±9.55	83.71±5.07	82.77±4.93	0.534
n (%) of MCV<80 fL	*7 (18.9)*	*5 (18.5)*	*7 (25)*	*9 (34.6)*	
RDW (%)	14.11±1.93	14.11±1.05	14.11±1.34	15.19±2.26	0.051
n (%) of RDW<15%	*5 (13.5)*	*4 (14.8)*	*4 (14.3)*	*7 (26.9)*	

Table 4. Fractional Iron Absorption: Absolute and Logarithmic Forms according to One-Way ANOVA

BMI Group	Lean (n=37)	Overweight (n=27)	Obese Class I (n=28)	Obese Class II (n=26)	ANOVA *p*-value
Absolute Fractional Absorption [%]	13.75±9.08	18.59±13.47	19.53±14.96	17.64±12.37	0.227
Log Fractional Iron Absorption	1.04±0.31	1.16±0.31	1.19±0.30	1.13±0.34	0.241

Both absolute form and logarithmic form of fractional iron absorption are not statistically significant using one-way ANOVA.

D. Iron Status Markers

As for markers of iron status, Table 5 shows that serum iron decreased from 76.89µg/ml to 69.19µg/ml comparing lean participants to obese class II participants. A similar scenario was applied to other iron markers like hemoglobin levels (12.91g/dl in lean group compared to 12.54g/dl in obese class II), and transferrin receptors saturation (17.14% in lean group compared to 13.71% in obese class II group).

Table 5. Iron Status Markers among Different BMI groups according to one-way ANOVA and Bonferroni post-hoc analysis

BMI Group	Lean (n=37)	Overweight (n=27)	Obese Class I (n=28)	Obese Class II (n=26)	One-way ANOVA *p*-value
FER (ug/L)	26.52±21.2	29.08±35.54	32.65±30.21	39.48±32.69	0.374
Hepcidin (ng/mL)	6.44±8.90	7.81±7.59	7.04±5.06	9.12±7.09	0.555
sTFR (mg/L)	6.39±2.92	6.40±2.60	6.54±2.42	7.92±5.54	0.305
Body Fe Stores (mg/kg BW)	2.78±3.74	2.55±4.21	3.10±4.00	3.23±4.98	0.931
Serum Iron (µg/mL)	76.89±29.03	73.07±40.58	79.93±33.00	69.19±27.91	0.644
TIBC(µg/dL)	470.81±83.46	504.78±55.35	504.50±87.75	521.31±64.21	0.056
TfR Saturation (%)	17.14±7.83	14.66±8.04	16.64±8.24	13.71±6.09	0.266

Moreover, ferritin and hepcidin who are considered as both iron status markers and inflammatory markers showed as well a similar increasing scheme as the previous markers that are mentioned above. None of the 4 BMI categories had a ferritin level that is low (<15.0ug/L). Furthermore, ferritin has increased sharply from 26.52ug/L in lean group reaching 39.48ug/L in obese class II group. As for hepcidin levels, the scenario was replicate showing an increase from 6.44ng/mL to 9.12ng/mL moving from lean group to obese class II group.

As for the other markers of iron status, they were elevated in the group of obese females class II such as body iron stores (2.78mg/kg BW in lean group compared to 3.23mg/kg BW in obese class II group), TIBC (470.81mcg/dl in lean group compared to 521.31mcg/dl in obese class II), and RDW (14.11% in lean group compared to 15.19% in obese class II group).

On the other hand, fractional iron absorption increased comparing the lean group to obese class II group moving from 13.75% to 17.64% respectively.

However, despite all the differences between BMI groups for all iron status markers, the difference in means for all iron status markers is not statistically significant (p-value<0.05)

E. Inflammatory Markers

On the other hand, the inflammatory status of our participants manifested by AGP, and CRP levels got worsened moving from group 1 of normal BMI to obese group of class II where lean group showed a mean of AGP equal to 0.81g/L and CRP equal to 2.43mg/L compared to 1.25g/L and 8.17mg/L respectively.

The mean difference in AGP and CRP among 4 BMI groups is statistically significant with p-value<0.05.

Although mean levels of CRP & AGP have been significantly different among different BMI groups, Table 6 asserts that the increase is not only a general scheme in the levels of individuals within each BMI group, but also the number of participants with levels of CRP and AGP above the normal cut-offs are also increasing moving up the BMI ladder.

Table 6. Descriptive statistics (mean ± SD) of Inflammatory Markers: CRP & AGP

BMI Group	**Lean (n=37)**	**Overweight (n=27)**	**Obese Class I (n=28)**	**Obese Class II (n=26)**	**ANOVA *p*-value**
AGP (g/L)	0.81±0.48[a]	1.07±0.41[ab]	1.23±0.46[b]	1.25±0.43[b]	0.000*
n (%) of AGP>1g/L	8(21.6)	13 (48.1)	16 (57.1)	19 (73.1)	
CRP (mg/L)	2.43±4.36 [a]	2.48±3.81[a]	3.88±2.97[a]	8.17±9.74[b]	0.000*
n(%) of CRP>5mg/L	4 (10.8)	4 (14.8)	8 (28.6)	14 (53.8)	

* Statistically significant (p-value<0.05)
* Groups having different subscripts are statistically different according to Bonferroni post-hoc analysis

Table 6 shows that the number of participants with high CRP levels i.e above 5mg/L increases from 10.8% to 53.8% going from lean group to obese class II respectively. As for high AGP levels (>1g/L), the percentage also has moved up from 21.6% in lean group to obese class II reaching 73.1%.

This shows that as BMI increases, inflammation gets worsened and this is manifested by inflammatory markers above the normal cut-offs.

Mean CRP has been majorly different comparing obese class II group with the normal BMI groups. This shows that CRP levels fail to show significant differences when obesity gradually increases, and it requires a significant difference in the degree of

obesity for CRP levels to be significantly differ.

As for mean AGP levels is only significantly different when comparing both obesity classes to normal BMI group. Overweight group did not show any significantly different results with neither normal BMI group nor obese groups. This shows that AGP is more sensitive to obesity classes than CRP since obese class I people succeeded to show a significant difference in their AGP levels when compared with those of lean people. Yet, CRP levels only showed differences when compared to obese class II people. So, at earlier obesity phases, AGP is a better obesity-sensitive marker of inflammation than CRP levels.

F. Correlations of Body Composition Markers with Inflammatory Markers

Table 7 shows that CRP and AGP are significantly correlated with all the body composition markers: lean body content (kg, %), fat content (kg, %), android and gynoid fat mass, visceral fat area and android/gynoid ratio.

On the other hand, hepcidin and ferritin are not as correlated with the DEXA markers as the first two inflammatory markers. This shows that CRP and AGP are more sensitive to body composition markers than hepcidin and ferritin. Furthermore, it shows that both location and quantity of fat affected CRP & AGP levels.

Ferritin was only significantly correlated with lean body mass and android/gynoid ratio which means that location of fat affects ferritin levels and not the quantity of fat in the body.

As for hepcidin, it wasn’t affected by any body composition marker.

Table 7. Correlation between Adiposity and Inflammatory Markers using Pearson's Correlation

Variable	CRP (mg/L)		AGP (g/L)		Hepcidin (ng/mL)		Ferritin (µg/L)	
	R	*P*-value	R	*P*-value	R	*P*-value	R	*P*-value
Lean Body Mass (Kg)	0.228	0.013*	0.263	0.004*	0.117	0.209	0.194	0.036*
Lean Body Mass (%)	-0.309	0.001*	-0.309	0.001*	-0.107	0.252	-0.024	0.793
Total Fat Mass (kg)	0.330	0.000*	0.291	0.001*	0.116	0.217	0.090	0.332
Percent Body Fat (%)	0.309	0.001*	0.308	0.001*	0.107	0.252	0.024	0.797
Android Fat Mass (kg)	0.325	0.000*	0.346	0.000*	0.144	0.123	0.128	0.169
Gynoid Fat Mass (kg)	0.272	0.003*	0.211	0.022*	0.092	0.327	-0.008	0.929
Android/Gynoid Ratio	0.253	0.006*	0.470	0.000*	0.148	0.112	0.194	0.035*
Visceral Fat Area (cm^2)	0.335	0.000*	0.374	0.000*	0.065	0.486	0.124	0.182

*Statistically significant with *p*-value<0.05

G. Correlations of Independent Variables with Fractional Iron Absorption among Different Four BMI Groups

Now after seeing how our independent variables affected when BMI increases and adiposity increases, the following tables show how their association with fractional iron absorption is.

It is important to mention that the logarithmic form of fractional iron absorption is used in order to get more accurate results since the variable fractional iron absorption is the main outcome of this study and doesn't follow normality.

Adiposity markers like visceral fat area, android fat mass, gynoid fat mass, percent body fat are statistically correlated with fractional iron absorption (Table 8).

Table 8. Correlation between Adiposity Markers and Log Fractional Iron Absorption

Variable	**Log Fractional Iron Absorption**	
	R	*P*-value
Lean Body Mass (Kg)	0.085	0.360
Lean Body Mass (%)	-0.213	0.020*
Total Fat Mass (kg)	0.193	0.037*
Percent Body Fat (%)	0.214	0.020*
Android Fat Mass (kg)	0.204	0.027*
Gynoid Fat Mass (kg)	0.220	0.017*
Android/Gynoid Ratio	0.190	0.039*
Visceral Fat Area (cm^2)	0.180	0.052

*Statistically significant with *p*-value<0.05

As for Table 9, it shows that only CRP was significantly correlated with log fractional iron absorption.

Table 9. Correlation between Inflammatory Markers and Log Fractional Iron Absorption

Depending Variable	**R**	***p*-value**
CRP(mg/L)	-0.199	0.030*
AGP (g/L)	0.063	0.495

*Statistically significant with *p*-value<0.05

Table 10 shows that log fractional iron absorption has been different among both groups. This shows that when comparing AGP levels in all the sample with fractional iron absorption, the difference wasn't significant (*p*-value = 0.495 in Table 9). Yet when the sample was stratified into normal and high AGP levels groups, the difference in fractional iron absorption appeared.

Table 10. Independent-t Test between AGP and Log Fractional Iron Absorption

Depending Variable	Independent Variable	*p*-value
Log Fractional Iron Absorption	Normal AGP (<1g/L)	0.039*
	High AGP (>1g/L)	0.043*

*Statistically significant with *p*-value<0.05

Table 11. Correlation between Inflammatory Markers and Log Fractional Iron Absorption

Depending Variable	R	*P*-value
Hepcidin (ng/mL)	-0.485	0.000*
Ferritin (µg/L)	-0.603	0.000*
Body Fe Stores (mg/kg BW)	-0.634	0.000*
sTfR (mg/L)	0.320	0.000*
TIBC (mcg/dL)	0.317	0.000*
TfR Saturation (%)	-0.391	0.000*
RDW (%)	0.361	0.000*
Serum iron (µg/dL)	-0.348	0.000*
Hemoglobin (g/dL)	-0.318	0.000*

*Statistically significant with *p*-value<0.05

H. Simple Linear Regression of Independent Variables with Fractional Iron Absorption

Table 12 illustrates using the simple linear regression model, that the independent continuous variables share a significant linear relationship with fractional iron absorption and this linear relationship is significant statistically (*p*-value<0.05). The only statistically-insignificant variable is visceral fat.

Table 12. Simple Linear Regression of Statistically Significant Correlations with Log Fractional Iron Absorption

Depending Variable	Log Fractional Iron Absorption					
	R	R Square	SE	Constant	*B*	*p*-value
Hepcidin (ng/mL)	0.485	0.235	0.625	2.963	-0.047	0.000*
Ferritin (µg/L)	0.603	0.363	0.585	3.057	-0.015	0.000*
Body Fe Stores (mg/kg BW)	0.634	0.402	0.567	2.915	-0.111	0.000*
sTfR (mg/L)	0.320	0.103	0.694	2.143	0.066	0.000*
TIBC (mcg/dL)	0.317	0.101	0.695	1.084	0.003	0.000*
TfR Saturation (%)	0.391	0.153	0.675	3.176	-0.037	0.000*
RDW (%)	0.361	0.130	0.684	0.445	0.150	0.000*
Serum iron (µg/dL)	0.348	0.121	0.687	3.179	-0.008	0.000*
Hemoglobin (g/dL)	0.318	0.101	0.695	5.026	-0.189	0.000*
Android Fat Mass (g)	0.204	0.041	0.718	2.256	0.000	0.027*
Lean Body Mass (%)	0.213	0.046	0.717	4.141	-0.028	0.020*
Total Fat Mass (kg)	0.193	0.037	0.719	2.154	0.000	0.037*
Android/Gynoid Ratio	0.190	0.036	0.720	1.512	1.169	0.039*
Body Fat (%)	0.214	0.046	0.717	1.368	0.028	0.020*
Gynoid Fat Mass (g)	0.220	0.048	0.716	2.031	0.000	0.017*
CRP(mg/L)	0.199	0.040	0.719	2.689	-0.024	0.030*

*Statistically significant with *p*-value<0.05

The linear relationship with log fractional iron absorption was the strongest in body iron stores where 40.2% of the change in fractional iron absorption is explained by the change in body iron stores and as body iron stores increases by 1mg/kg BW, fractional iron absorption decreases by 0.111%.

I. Multiple Linear Regression of Independent Variables with Fractional Iron Absorption

Table 13 shows that after combining the three independent variables together, that were significantly linear with fractional iron absorption changes when taken solely, only body iron stores and CRP succeeded at maintaining the linear relationship statistically significant. The below table shows that as body iron stores increase by 1 mg/kg BW, fractional iron absorption decreases by 0.046%. Furthermore, as CRP

increases by 1 mg/L, body iron stores decreases by 0.01%. This shows that body iron stores are a stronger factor affecting fractional iron absorption, yet it co-existed with CRP levels being the only significant independent variables affecting fractional iron absorption is body iron stores.

Table 13. Multiple Linear Regression of Dependent Variables with Log Fractional Iron Absorption

Variables	**B**	**SE**	***p*-value**
(Constant)	0.648	0.334	0.055
Body Fe Stores (mg/kg BW)	-0.046	0.005	0.000*
CRP (mg/L)	-0.010	0.004	0.017*
Android (A) Fat mass (g)	$-4.26*10^{-5}$	0.000	0.622
Gynoid (G) Fat mass (g)	$3.43*10^{-5}$	0.000	0.411
Percent Body Fat (%)	-0.002	0.009	0.786
Android/Gynoid Ratio	0.633	0.413	-.128
VAT Area (cm^2)	0.001	0.001	0.524

*Statistically significant with *p*-value<0.05

This would bring our statistical analysis to a new area of thinking where the effect of the visceral fat alone, or the effect of android fat compared to gynoid fat is exaggerated. Moreover, the effect of inflammatory markers and worsened inflammatory status is also overrated. None of the latter showed a significant effect on fractional iron absorption and the sole main factor that remained significant till the end is body iron stores.

CHAPTER V

DISCUSSION

A. Interpretation

Obesity is a condition that puts the individual in a state of low-grade inflammation. Adipocytes release adipokines and inflammatory mediators that trigger the reticulo-endothelial cells into capturing iron, leading to lower iron levels available for erythropoeisis thus causing hypoferremia. In addition, the secretion of hepcidin, a hepatoprotein, and its release from hepatocytes increases as a result of the elevated inflammatory markers leading to decreased iron absorption at the level of the small intestine acting on ferroportin 1. Severe scenarios of hypoferremia lead to the development of the so-called "anemia of chronic disease". Several studies have showed a negative correlation between iron markers like ferritin, transferrin and hepcidin with BMI and inflammatory statuses (Khan *et al*., 2016; Nairz *et al*., 2016; Schmidt, 2015; Yanoff *et al*., 2007; Nemeth *et al*., 2003).

Several studies showed that obese and overweight subjects have lower iron statuses though their iron intake is adequate (Tussing-Humphreys *et al*., 2009; Menzie *et al*., 2008; Yanoff *et al*., 2007). It is thought that the low-grade inflammation caused by adiposity causes this hypoferremia. However, it is not clear whether this inflammatory status is related to the quantity of fat in the human body or the location of fat or a combination of both factors.

The objective of the study covered in this thesis is to check whether elevated inflammatory levels caused by adiposity levels is caused by increased absolute fat mass in the body of our pre-menopausal overweight and/or obese females of childbearing age

or it is due to altered android/gynoid fat ratio.

Although A/G ratio wasn't altered much between BMI groups, visceral fat has been statistically different among all BMI groups. VAT area has increased moving from lean category to obese class II category. Even when the difference in mean android/gynoid ratio between obese class I and obese class II participants became insignificant, the change in mean visceral fat area remained statistically different. This shows that regardless of the proportional increase in both android and gynoid areas, visceral fat is more affected with the increase in adiposity.

A study conducted on Chinese lean females of childbearing age, their average percent body fat was 31.6%, 18.5kg average body fat mass, 1.8kg and 3.2kg as average android and gynoid fat respectively. Their A/G ratio was 0.6 (Fu *et al*., 2014).

Another study conducted on lean healthy Caucasian females showed that their mean percent body fat was 31.75%, 19.70kgs as their total body fat mass, 1.21kgs as android fat and 3.79kgs as their gynoid fat. It is important to mention here that the mean age of the sample was around 32 years which is similar to the age of our sample of study. Moreover, both lean categories of our study and Miazgowski's study have mean waist circumference of around 75cm (Miazgowski *et al*., 2014).

Our adiposity markers for our lean category were as follows: 38.09% mean percent body fat, 24kg mean total fat mass, 1.44kg and 4.79kg for android and gynoid fat mass average respectively. Average A/G ratio was 0.8.

This shows that even the individuals of normal weight in our sample, of the same age, tended to have higher adiposity level than lean individuals of other origins. Both android and gynoid areas of our participants tend to be higher than levels of lean females of different origins and the fat is more likely to be peripherally located.

Moreover, lean body mass increased significantly among different BMI groups

yet it reached a plateau between obese class I and obese class II where the difference was statistically insignificant. This plateau effect among the two obese classes has been observed in another body composition marker: percent body fat.

As for hematological results, they show that adiposity didn't affect hemoglobin levels, mean corpuscular volume and red cell distribution width significantly (p=0.346, 0.534, 0.051 respectively). Mean hemoglobin levels of our sample of study slightly decreased yet the decrease wasn't statistically significant. Hemoglobin levels of normal-weighted females in a Swiss study conducted by ETH Zurich were 13.5g/dL which wasn't different moving to overweight and obese individuals (Cepeda-Lopez *et al*., 2018). This shows that the baseline of our study is slightly lower in terms of hemoglobin levels yet the difference in hemoglobin levels among different BMI groups wasn't statistically different (*p*-value=0.346>0.05).

Moreover, the inflammatory markers AGP and CRP levels were significantly different among different BMI groups. 8% of the normal-weighted individuals had a CRP level above 5mg/L where this number increased to 19% in the obese class II category.

A study conducted over 8 countries on a similar sample of overweight/obese pre-menopausal females showed that their AGP levels ranged between 1.1 to 3g/L whereas their CRP levels ranged between 2.2mg/L to 3.1mg/L. This shows that our sample had a lower AGP yet a higher CRP level (Williams *et al*., 2016). Another Swiss study conducted by Dr. Michael Zimmerman showed a 0.95g/dl mean AGP levels for both overweight and obese females compared to 2.68mg/dL as CRP mean level (Cepeda-Lopez *et al*., 2018).

One more study conducted on Cuban overweight/obese women of childbearing age showed a average of 0.80g/L and 0.90mg/L for AGP & CRP respectively (Pita-

Rodriguez *et al*., 2017). After looking at results from other studies conducted on other populations, we can see that the inflammatory profile of our sample is slightly higher which may be explained by the higher adiposity observed in our sample of study. Moreover, CRP and AGP levels were significantly correlated with all body composition markers unlike hepcidin and ferritin levels. This shows that CRP and AGP are more sensitive to the change in body composition markers in general, and adiposity markers in specific, than hepcidin and ferritin levels. Yet, inflammatory markers didn't significantly affect iron absorption level which is an observation that is not far from other previously conducted studies. According to Cheng *et al*., neither inflammation nor hepcidin levels were sufficient factors in inducing lower iron status or absorption even in obese class II individuals (2013).

Regarding iron status markers, they weren't significantly altered due to the change in BMI statuses. According to Cepeda-Lopez, overweight and obese females in a study conducted in Switzerland showed an average hemoglobin level around 13.7g/dL, sTfR mean level around 6.84mg/L, body iron around 5.70mg/kg body weight, mean serum hepcidin of 11.92ng/mL (2018).

Our obese class II category illustrates hepcidin levels of an average of 9.12ng/mL, body iron stores of 3.23mg/kg body weight, hemoglobin mean level of 12.54g/dL and means sTfR level of 7.92mg/L. As for transferrin saturation, the average for obese females was 13.71% in our study compared to 26.8% in the study mentioned earlier (Cepeda-Lopez *et al.*, 2018). The comparison of our results with the results of another study shows that our sample has a lower iron status which may be explained by the higher inflammatory status they have compared to other samples of other studies.

Our results prove that when iron levels are low which is manifested by low fasting serum iron levels, low body iron stores, low transferrin receptor saturation, low

hemoglobin levels and low ferritin levels, the body reacts to this scenario by increasing the absorption of iron at the duodenal level, increasing the number of transferrin receptors at the hepatic level and increasing serum total iron binding capacity with the intention of restoring iron levels to normal.

Digging into the main outcome of this study, which is fractional iron absorption, its absolute value wasn't statistically different among 4 obesity groups. As for log fractional iron absorption, it also remained insignificant. A study conducted on lean Thai women of the same age group shows that their mean fractional iron absorption is around 10%, their mean ferritin levels were around 32 ug/L and their CRP levels were around 0.61mg/L (Zimmerman *et al*., 2008). As for our study, mean absolute fractional iron absorption level for the lean group was around 14% with ferritin levels around 27ug/L and CRP levels around 2.43mg/L. This shows that our sample had a higher baseline inflammation, lower baseline iron status and higher baseline fractional iron absorption.

A close scenario has also been observed in another study conducted on normal, overweight and obese females of childbearing age show that in lean group, serum ferritin level is on average 50.6 ug/L, mean CRP levels is 1.05mg/dL, mean AGP levels around 0.79g/L and baseline hepcidin average is 9.20ng/mL (Cepeda-Lopez *et al*., 2015). Their baseline iron absorption was 19% on average in normal-weighted group. This shows that our lean category considered as our baseline value, has a higher inflammatory status (hepcidin=6.44ng/mL, CRP=2.43mg/dL, and AGP=0.81g/L), lower iron status (ferritin=26.52 ug/L) and lower fractional iron absorption (13.75%).

Looking at results of the correlation between adiposity markers and fractional iron absorption, fractional iron absorption was significantly affected positively by all body composition markers except for lean body mass and visceral fat area. Moreover,

percent lean body mass was negatively correlated with fractional iron absorption. Our findings are unexpected since it is known that the more metabolically-active visceral fat worsens the inflammatory status thus expected to decrease the absorption of iron. Furthermore, increase in android fat mass has been proven to be linked to elevated VAT in previous studies. This can be explained by the fact that the inflammatory profile caused by elevated adiposity in our participants wasn't high enough to show effect on fractional iron absorption.

On the other hand, hepcidin has been recently the interest of research due to the fact that when iron levels are elevated in the body, manifested mainly as high levels of ferritin in the liver, hepatocytes increase the synthesis of hepcidin transcriptionally to preserve homeostasis which subsequently elevates hepcidin levels in the circulation. The reason behind this is to decrease iron levels by decreasing iron absorption at the intestinal levels via binding to ferroportin 1 which will be internalized and degraded. In addition to hepcidin's action at the level of the mucocyte, it as well decreases the release of iron at the levels of macrophages which will prevent more iron available for erythropoeisis (Andrews, 2008).

Our results do not contradict with what's been proved before. They show that fractional iron absorption and hepcidin levels are significantly negatively correlated (*p*-value<0.05).

This shows that hepcidin measurement is a good indicator for iron status measurement. Indicators for iron status are used because the gold standard method to measure iron absorption requires researchers to have high technical skills, is sophisticated and expensive. Hepcidin levels can give a good indicator and the test is cheaper, more widely available and more practical.

However, one important issue that stays unrevealed and has been seen as an

obstacle in the analysis of the results of this study is the official cut-offs for desirable hepcidin levels that remain unclear. This is why ferritin is a better indicator for researchers and healthcare professionals to follow and use as an indicator for iron status because they have globally acknowledged cut-offs that are set by WHO. This stays as a route to be focused on by researchers in order to apply the same effort into finding the appropriate cut-offs for hepcidin to be more easily used as a reliable criterion to indicate an individual's iron status.

After combining variables together, only body iron stores remained the main independent variable affecting fractional iron absorption is body iron stores. The study results might have been different more flagrant if the participants have suffered from iron deficiency or anemia. Yet, this is not the case in our selected sample of study where around 80% of the sample chosen is composed of participants that do not suffer from anemia or iron deficiency (n=101 with sFTR>8.3mg/L and n=94 with Hb>12g/dl).

This would bring our statistical analysis to a new area of thinking where the effect of the visceral fat alone, and the effect of android fat compared to gynoid fat is exaggerated. None of the latter showed a significant effect on fractional iron absorption and the sole main factor that remained significant till the end is body iron stores.

B. Study Limitations

However, it is important to mention that the results of this study are not enough to eliminate this effect from our hypothetical assumptions since this study has also its limitations that would prevent it from highlighting the possibly significant nuances in results that exist but went missed.

- This effect would have been further highlighted if the sample size was bigger. However, this was hard to perform due to the frequent visiting times of the

participants and the costs of the tests that have been done which leaves increasing sample size an unpractical option to apply.

- Commitment of the participants to the experiment days and their compliance to the fasting hours, no caffeine or exhaustive physical activity one day prior to the test which are pre-experimental days must, can also be a limitation because how the participants behave can affect the obtained blood sample results.

- Furthermore, it is important to mention that in the Lebanese population where this study was conducted, it was hard to find exclusive peripheral obese females. Most of the females that were peripherally obese had also fat in the central area >43% which was enough for them to also be considered centrally obese. This would also shed the light on the need for new population-specific cut-offs to have proper categorization of individuals into peripherally or centrally obese. The cut-offs that are nowadays existing cannot be applied to the studied population.

- The genetic pool from which the sample was selected might also be a factor in showing closer results than others. A stratification of the Lebanese areas from which the sample is collected would have given us an elimination of this possible confounder.

- Moreover, it is crucial to mention that even if the participants were obese; they were metabolically healthy, suffering from no diseases and are not on any kind of medications. Individuals with diagnosed diabetes (HbA1c>6.5%) were excluded from the study. This would also be a possible factor into having weaker results. A new interesting possible hypothesis for a new study is whether a typical sample of obese and overweight pre-menopausal females but metabolically unhealthy (diagnosed with obesity-related comorbities eg. Diabetes Mellitus) would show similar results to the ones we had in this study or not.

On the other hand, despite of the limitations this study has, it is important to

give it the credit by mentioning its strengths as well.

C. Study Strengths

The study design on its own is a point of strength because it is a controlled non-randomized trial will where we are controlling for the exposure, the latter being the ingestion of a known amount of glucose and iron and labelled ^{57}Fe in order to study their absorption rates according to different states of body adiposity distribution, and level.

The sampling selection method is snowballing which shows that researchers had no interest or benefit in selecting those participants to volunteer in the study.

In addition, it is highlighting a topic that is yet unclear in research, which is the effect of different fat location on inflammatory levels and iron absorption levels, which makes it clinically valuable. In addition, it might be the door to solving underlying side effects for systematic iron supplementation that is being practically applied to all iron-deficient individuals regardless of their adiposity status.

Furthermore, the gold standard method for measuring fractional iron absorption with a labeled isotope is used which is not the common assay used in studies interested in measuring iron status and iron absorption levels. This gold standard method, despite of being the best measurement, is not the chosen by researchers because of its high cost and the budget limitation that most research studies are subject to. Furthermore, the labelled ^{57}Fe is rare to find which makes it the harder method to use.

In addition to this, it was a study conducted over 2 years while the researchers were trying to ensure the most precision and accuracy possible when it comes to the inclusion/exclusion criteria, participants showing up on exactly the 14^{th} day (experiment day #2) otherwise they will be dropped out of the study to prevent inaccurate results.

Moreover, proper machines calibration and maintaining the best environment for the collected blood samples at proper storage temperature in order to ensure the RBCs are kept in their best state are also points of credibility for our results.

CHAPTER VI

CONCLUSION

Our results show that despite the increase in adiposity among different BMI groups, the increase was proportional between android and gynoid areas. As for the A/G ratio among different BMI groups, the difference didn't cause a significant effect on fractional iron absorption. Yet, visceral fat remained on an increasing pattern among all BMI groups. The latter shows that the location of fat didn't have a significant effect on iron status when the amount of fat itself did.

As for body composition markers, they changed significantly among different BMI groups, yet they reached a plateau when moving from obese class I group to obese class II group. This plateau has been observed in lean body mass, percent body fat, and A/G ratio.

As for inflammatory markers, only CRP and AGP were dependent on body composition markers compared to hepcidin and ferritin yet they showed no significant effect on fractional iron absorption.

Moreover, our lean category showed a higher inflammatory profile, lower iron status and lower iron absorption compared to other lean groups of similar age groups in other studies conducted on populations of different origins and geographical locations.

Furthermore, the only factor that remained as the only determinant on fractional iron absorption was body iron stores.

We can conclude that obesity on its own is not enough of an indicator for iron absorption levels. It is crucial to keep in mind that the metabolic health of an individual remains the most important factor regardless of his weight and adiposity level. On the

opposite side of the spectrum, even a non-obese metabolically unhealthy individual can show altered blood lipid, impaired glucose homeostatic control and iron absorptive state that might be worse than the ones observed a metabolically healthy obese or overweight individual. The latter hypothesis needs further backup which calls for more research to be invested in this area of interest.

Last but not least, further studies are highly needed to be conducted while solely focusing on the effect of the fat cell size instead of fat location or the adiposity levels, on inflammatory levels and subsequently on iron absorption.

APPENDIX I

QUESTIONNAIRE IN ARABIC

الاسم: رقم المشارك: التاريخ:

العمر: الجنس:

الوضع المدني: المهنة:

عنوان السكن:

الطول: الوزن: قياس الخصر: مؤشر البدانة:

(تؤخذ القياسات من قبل الباحث و من ثم تعبئ المعلومات من قبله)

الرجاء الاجابة عن الأسئلة التالية:

1- هل تعاني من أي من الأمراض المذكورة أدناه:
   a. السكري
   b. أمراض القلب
   c. ارتفاع ضغط الدم
   d. ارتفاع الدهون في الدم
   e. فقر الدم
   f. أمراض أخرى:.....

2- هل خضعت لعمليات جراحية في السنوات الخمس الماضية؟
   ☐ كلا ☐ نعم (حدد).....

3- هل خسرت أو كسبت أكثر من 3 كغ في الأشهر الثلاثة الماضية؟
   ☐ كلا ☐ نعم

4- هل تأخذ أي أدوية في الوقت الحالي؟
   ☐ كلا ☐ نعم (حدد).....

5- هل أنت مدخن(ة)؟
   ☐ كلا ☐ نعم (حدد عدد السجائر في اليوم).....

6- هل تتناول شرب الكحول؟
☐ كلا
☐ نعم (حدد عدد الكؤوس في اليوم).....

7- هل كنت معتمد(ة) على الأدوية في السنوات الخمس الماضية؟
☐ كلا
☐ نعم

8- هل تأخذ أي مكملات غذائية؟
☐ كلا
☐ نعم (حدد).....

9- هل تتناول أي نوع من الحديد بالإضافة الى الموجود في طعامك؟
☐كلا
☐ نعم (حدد النوع و الكمية).....

10- هل تمارس التمارين الرياضية؟
☐ كلا
☐ نعم (حدد النوع و المدة الزمنية).....

11- هل أنت مسؤولة أو تشاركين في الشق المادي في المنزل؟
☐ كلا
☐ نعم (حدد النسبة % التقريبية).....

12- هل لديك أولاد؟
☐ كلا
☐ نعم (حدد عدد الأولاد).....

13- هل تشغل وظيفة بشكل منتظم؟
☐ كلا
☐ نعم

14- إذا أجبت بنعم على السؤال السابق، الرجاء إختيار إحدى الخانات المناسبة لمعاشك الشهري
أ- 500$ -1،000$
ب- 1و000$ -1،500$
ت- 1،500$ -2،000$
ث- 2،000$ -2،500$
ج- 2،500$ -5،000$
ح- أكثر من 5،000$

APPENDIX II

QUESTIONNAIRE IN ENGLISH

Name: **Subject number:** **Date:**

Age: **Gender:**

Civil status: **Profession:**

Address:

Height: **Weight:** **WC:** **BMI:**
(All filled by the investigator after taking the measurements)

Please answer the following questions:

1. **Do you suffer from one or more of the following?**
   a. Diabetes
   b. Heart diseases
   c. Dyslipidemia
   d. Hypertension
   e. Iron deficiency/ iron deficiency anemia
   f. Other: _____

2. **Did you undergo any surgery in the last 5 years?**
   ☐ No ☐ Yes (specify : _____)

3. **Did you lose or gain more than 3 Kilograms in the last 3 months?**
   ☐ No ☐ Yes

4. **Are you currently taking any medication?**
   ☐ No ☐ Yes (specify : _____)

5. **Are you a smoker?**
   ☐ No ☐ Yes (specify number of cigarettes per day: _____)

6. **Do you drink alcohol?**
   ☐ No ☐ Yes (specify average number of drinks per week _____)

7. **Have you been dependent on the use of drugs in the past 5 years?**
   ☐ No ☐ Yes

8. **Do you take any nutritional supplement?**
   ☐ No ☐ Yes (please specify _____)

9. **Do you take any iron supplement?**
   ☐ No ☐ Yes (please specify name and quantity_____)

10. **Do you do any exercise?**
    ☐ No ☐ Yes (please specify type, duration and frequency_____)

11. **Do you participate in the financial spending at home?**
    ☐ No ☐ Yes (please specify an approximate % _____)

12. **Do you have kids?**
    ☐ No ☐ Yes (please specify how many_____)

13. **Do you work?**
    ☐ No ☐ Yes (please specify how many_____)

14. **If you answered yes to the previous question, pick a range of your earned salary**
    a. $500-$1,000
    b. $1,000-$1,500
    c. $1,500-$2,000
    d. $2,000-$2,500
    e. $2,500-$5,000
    f. More than $5,000

APPENDIX III

ARABIC CONSENT FORM

Institutional Review Board
American University of Beirut

13 MAR 2017

RECEIVED

عنوان البحث: العلاقة بين السمنة والالتهابات ونسبة السكر في الدم و تأثيرها على امتصاص الحديد: مقارنة بين السمنة المركزية والطرفية.

إسم الباحث: د. عمر عبيد/ قسم التغذية و علم الطعام/ الجامعة الأمريكية في بيروت.

الباحثين المساعدين: د. باسم صفدي، د. مروان رفعة، د. هلا غطاس، د. عماد الطفيلي

منسقي البحث: كارلا الملاح

العنوان: الجامعة الأميركية في بيروت، شارع الحمرا، بيروت – لبنان 01-350000

مكان إجراء البحث: الجامعة الأميركية في بيروت – المركز الطبي و كلية الزراعة
مركز طبي: مؤسسة رفيق الحريري الطبية

أنت مدعو(ة) للمشاركة ببحث علمي سيجرى في الجامعة الأميركية في بيروت. الرجاء أن تأخذ(ي) الوقت الكافي لقراءة المعلومات التالية بتأن قبل أن تقرر(ي) إذا كنت تريد(ين) المشاركة أم لا. بإمكانك طلب إيضاحات أو معلومات إضافية عن أي شيء مذكور في هذه الإستمارة أو عن هذه الدراسة ككل من طبيبك.

أ) هدف هذا البحث: إن الوضع المتدني للحديد عند الأشخاص الذين يعانون من السمنة أو زيادة الوزن المفرطة أصبح شائعاً في الأونة الأخيرة، و قد تم ربطه في معظم الأحيان بنوعية الأطعمة المستهلكة من قبل هذه الأشخاص، إلا أن بعض الدراسات تشير الى عدم إرتباط معدل الحديد المنخفض بالطعام المستهلك، إنما درجة إمتصاصه هي التي تلعب الدور الأساسي في التأثير على معدلاته. و من ضمن الأمور التي تؤثر على مستوى الحديد هي وجود نسبة مرتفعة من مؤشرات الإلتهاب في الدم، و هي متواجدة بشكل مزمن عند هذه الأشخاص، و بالتالي، فقر الدم الذي تعاني منه هذه الأشخاص يشبه فقر الدم الملحوظ في حالات الأمراض المزمنة. لذا الهدف من هذه الدراسة هو دراسة تأثيرات اثنين من الأنواع الأكثر شيوعا من السمنة (المركزية مقابل الطرفية) على امتصاص الحديد، والالتهابات، ونسبة السكر في الدم لدى النساء قبل انقطاع الطمث، وتحديد حالة واضحة من نقص الحديد في حالات السمنة.

ب) عملية البحث عن مشاركين: عملية التوظيف في هذه الدراسة تنطوي على الاتصال بالأطباء في عدة أقسام من المركز الطبي للجامعة الأميركية في بيروت و المراكز الصحية في مؤسسة رفيق الحريري الطبية، بالإضافة إلى الاتصالات الشخصية. ، يليه بعد ذلك لقاء مباشر مع الطبيب المعني بالأمر. بعد الحصول على موافقة الأطباء ، يقوم فريق الباحثين بزيارات الى العيادات الطبية من أجل البحث عن مشاركين محتملين. عندها، سيتم شرح هذه الدراسة لهم، وبالتالي، سوف يطلب من المهتمين بالمشاركة في الدراسة و الذين يتناسبون مع معايير الاشتمال، على التوقيع على استمارة الموافقة و ملء استبيان حول وضعهم الاجتماعي والاقتصادي والديمغرافي والصحي.

ج) وصف الإجراءات والمشروع: خلال هذه الدراسة، سوف يطلب منك الحفاظ على العادات الغذائية والنشاطات البدنية المعتادة بأكملها، وتجنب استهلاك الكحول، فضلا عن ممارسة التمارين الرياضية المفرطة قبل 24 ساعة من الحضور الى المركز. وتشمل معايير الاستبعاد: الأشخاص الذين يعانون من الأمراض التي قد تؤثر على الدم، مثل الثلاسيميا، بالإضافة إلى وجود أي إلتهابات، و تناول الحديد كمكمل غذائي.

التحضير الوحيد الذي عليك القيام به هو أن تأتي بعد حالة الصيام لمدة 12 ساعة ووقف تناول أي مكمل غذائي. كمرحلة أولى، سوف يطلب منك المجيئ الى قسم التغذية في كلية الزراعة / الجامعة الأميركية في بيروت، حيث سيتم قياس الطول، الوزن، محيط الخصر، بالإضافة الى خضوعك لفحص على مكنة تحليل المقاومة البايوكهربائية (BIA) و الذي يرتكز على ميزان يبعث موجات بايوكهربائية في الجسم و بالتالي يستطيع تحديد تكوين الجسد (عظم، دهون، عضل، مياه.. و أين تواجدها في الجسم) و الماسح الضوئي للعظام (DEXA) و الذي بدوره يرتكز على استلقاء الشخص على الماسح من دون الحراك و من ثم يقوم الجهاز بالمسح الضوئي للجسد و بالتالي يستطيع تحديد نسبة و أماكن توزيع الدهون، العضل، العظم و المياه في الجسم بالإضافة الى كمية كل منهم، و سوف يطلب منك ملء استبيانات حول صحتك ووضعك الاجتماعي

والاقتصادي. إذا كنت تتطابقي مع جميع المواصفات المطلوبة، عندها سوف يتم تحديد موعد الزيارة الأولى الى وحدة البحوث المركزي (CRU) / مستشفى الجامعة الأميركية، حيث سيتم أخذ عينة من الدم في حالة الصيام، ومن ثم سوف تعطين وجبة مكونة من خبز، زبدة و عسل، و هي تحتوي على 6 ملغ من نظير الحديد المسمى ^{57}Fe. سوف يطلب منك عدم تناول الطعام أو الشراب لمدة 3 ساعات بعد تناول وجبة الاختبار من أجل الحد من تأثير المواد الغذائية الإضافية على امتصاص الحديد. بعد ذلك، سوف يحدد موعد ثاني للقدوم الى وحدة البحوث المركزي (CRU) / مستشفى الجامعة الأميركية بعد 14 يوماً بالظبط. خلال هذا الموعد، سوف يتم أخذ عينة دم على الريق، و من ثم، سوف يتم إعطاؤك شراب يحتوي على 75 غرام من السكر و 100 ملغ من الحديد، وسيتم بعدها سحب عينة دم أخرى بعد ساعتين من تناول الشراب.
كلفة البحث مغطاة من قبلنا و قبل مركز ETH Zurich العلمي في سويسرا، لذلك لا يتوجب عليكم دفع أي شيئ من أجل المشاركة.

د) **المدة**: إن الوقت المقدر لانهاء البحث هو أسبوعين، حيث على المشاركة في البحث الحضور مرة من أجل التقييم و التأكد من مطابقتها لجميع معايير الدراسة، و هذه الزيارة تحتاج الى قرابة النصف ساعة. و من بعدها، عليها الحضور يوم واحد من كل أسبوع من مدة الدراسة إلى مكان الاختبار. مدة الزيارة الأولى تحتاج الى قرابة الساعة. سوف يطلب منك العودة الى مكان الدراسة بعد أسبوعين (14 يوماً بالظبط) من أجل سحب العينتين الأخرتين من الدم، هذه الزيارة بحاجة الى حوالي ال 2 الى 3 ساعات من وقتك. يمكنك الانسحاب من البحث في أي وقت. إن أردت التوقف عن المشاركة، ما من عقوبة تفرض عليك ولن تخسر أي من الفوائد التي تملكها وقرارك لن يؤثر على أي علاقة مستقبلية مع الجامعة الأمريكية في بيروت.

ه) المخاطر والمضايقات والفوائد: لقد أجرينا مسبقا عدة تجارب مستخدمين مقدار الجرعة نفسه من الحديد و السكر ولم نتلقى اي شكاوي من تأثيرات جانبية أو انزعاج، ولكن هناك احتمال حدوث مخاطر غير متوقعة. مشاركتك في هذه الدراسة قد يكون لها اثار جانبية ثانوية. سوف نطلب منك أن تجلسي بشكل مريح على سرير. تضع ممرضة مؤهلة حقنة مثبتة (أي أنبوبة بلاستيكية صغيرة) في وريد الساعد من أجل سحب الدم و إعطاؤك جرعة الحديد، و سوف تسحب الحقنة المثبتة بعد الإنتهاء من إدخال الحديد الى الوريد، أي بعد 30 دقيقة. أنواع الحديد التي ستستخدم لم يعرف عنها بأنها خطرة أو تؤذي الصحة. فحصي الماسح الضوئي و تحليل المقاومة لا يشكلان أي خطر على الصحة و هما فحصين سريعين. و لكن قد تكون هناك مخاطر لا يمكن التنبؤ بها. لا يمكنك المشاركة في هذه الدراسة في حال كنت حامل. إذا من الممكن أن تحبلي، سيتم إخضاعك لفحص كشف الحمل قبل إخضاعك للأشعة. إذا من الممكن أن تكوني قد حبلت في الأربعة عشر يوم الماضية عليك إبلاغنا لأن فحص كشف الحمل غير دقيق خلال هذين الأسبوعين. بالرغم من عدم وجود أي مخاطر ظاهرة أو معروفة من كمية الأشعة التي ستتعرضين لها خلال هذه الدراسة، ألا و أنه من الممكن وجود عوارض جانبية لاحقة لا يمكن التنبؤ بها. لا يمكنك المشاركة في البحث إن كنت حامل. و إن لم تكوني، فسوف تسألين عن تاريخ اليوم الأخير من آخر العادة الشهرية و سوف تخضعين للماسح الضوئي و البايوكهرباتي خلال الأيام العشرة الأولى من اليوم الأول للعادة الشهرية السابقة. بالإضافة، سوف نقوم بإختبار سريع للحمل فقط من أجل التأكد من عدم وجوده.

المخاطر التي يمكن التنبؤ بها:

- من المحتمل أن تتراوح المخاطر من إحمرار بسيط للجلد إلى التهاب محلي لوريد الساعد، تكتل الدم في مكان سحب الدم، أو حالة الإغماء عند بعض الأشخاص.
- على الصعيد النفسي من الممكن أن يشعر بعض المشاركين بالقلق عند أخذ عيّنات الدم أو وضع الحقنة.

سيتم إتخاذ التدابير التالية لتخفيف المخاطر المذكورة أعلاه:

- سيتم شرح تفصيلي لبروتوكول الدراسة والفحوصات التي ستخضع لها عند المقابلة لضمان راحتك.
- ستجرى جميع الفحوصات حسب البروتوكول الطبي.
- لتخفيف المخاطر المتعلّقة باستخدام الحقنة، سوف تعقّم منطقة الجلد جيّداً بالسبيرتو قبل وضعها من قبل ممرضة مؤهلة. سوف تتأكد الممرّضة من وضعها الصحيح وتقوم بجمع عيّنات الدم بالطريقة السليمة. ستكون جميع الأدوات التي تستعمل معقّمة. ستُسحب الحقنة فقط من الممرضة. عندما يتم سحب الحقنة ، يُنظّف الجرح بالسبيرتو والبيروكسيد ثم تُوضع لزقة معقّمة.
- سيُطلب منك الإبلاغ عن أي إزعاج أو تغيير لون في جلدك.
- إن استعمال الإبرة المثبتة أفضل من الإبرة التي يجب وضعها وسحبها عدة مرّات لأنها تسبب أقل إزعاج عند أخذ عيّنات متكررة. سوف تشعر بالقليل من الألم عند إدخال الأبرة و لكن لن تشعر بشيئ خلال عملية سحب الدم.

تناول الحديد بكميات كبيرة يؤدي في العادة الى الشعور ببعض الأوجاع و الإلتز عاجات في الجهاز الهضمي، كالغثيان و تشكل الغازات. و لكن بالكميات التي سوف يتم تناولها خلال هذا البحث، يفترض عدم الشعور بأي من تلك العوارض.

فوائد هذه الدراسة: من الممكن أن تساهم نتائج هذا البحث في الدراسات المهتمة بفقر الدم الناتج عن السمنة المفرطة. فاذا استطعنا عبر هذه الدراسة من معرفة كيفية و مدى تأثير السمنة، و نسبة الإلتهابات و السكر في الدم على معدل امتصاص الحديد، سيمكننا ذلك من تطبيق فحص دم جديد يقوم به الأشخاص قبل البدء بتناول المكمّل الغذائي الذي يحتوي على الحديد، لتشخيص و تحديد مدى صحّة و دقّة فقر الدم في حال البدانة، من أجل التأدم من حاجته لهذا المكمّل .

و) السرية: لتأمين سرية إجاباتك، إسمك والمعرفات الأخرى لن تكون معلقة مع أجوبتك لضمان السرية. جميع المعلومات والمدونات ستحفظ في غرفة مغلقة أو حاسوب لديه رمز سري. الوصول إلى المعلومات مسموحٌ فقط للباحث الأساسي والباحثين الذين يعملون مباشرةً على الدراسة. جميع المعلومات ستدمر بشكل مسؤول من بعد الوقت المطلوب .سيحافظ على سريتك في جميع المعلومات المكتوبة والمنشورة عن نتائج هذا البحث. لن يستعمل إسمك أو أي معلومة متعلقة بهويتك في تقاريرنا أو مقالاتنا المنشورة.من الممكن أن توجد ظروف حيث يجب نشر معلوماتك السرية. مثلاً يمكن للمعلومات الشخصية المتعلقة باشتراكك أن تعطى لمجلس المراجعة المؤسسية في الجامعة الأمريكية في بيروت إن طُلبت و للجان الأخلاق المهنية المستقلة، ومفتشين من الإدارات الحكومية المنظمة، مكتب حماية البحث الإنساني للولايات المتحدة أو أي وكالة تنظيمية فدرالية أو دولية أخرى، أو راعي البحث، إن وجد أو أي وكالة تسند البحث.

ز) التعويض / الحافزة: سوف يتم دفع مبلغ $50 أميركي، مقسوم على مرحلتين، من أجل تغطية التنقلات من و الى مركز البحوث على مدّة الدراسة، و لكن لن يكون هناك اي تعويضات مالية لتغطية التكاليف في حال لم تغطّيها شركات التأمين الطبي او الإستشفائي او أي برامج حكومية إذا حصل أي عارض سلبي من جراء المشاركة في هذه الدراسة. بالإضافة الى ذلك، سوف تحصلين على نتائج فحوصاتك الأولية (فحص الدم و تحليل الجسم) عند عودتك من أجل الفحص النهائي بعد أسبوعين.

ح) الدفع للإصابات ذات صلة بالبحث: ان المركز الطبي في الجامعة الأميركية في بيروت سوف يغطي تكاليف العلاج في المركز للعوارض الطبية السلبية الناجمة مباشرة عن الأدوية و/أو الإجراءات الطبية الخاصة بهذه الدراسة البحثية. في ما عدا ذلك، لن يقوم المركز الطبي بتغطية تكاليف العناية الطبية لأية حالة أو مُشكلة مَرَضية.
إن تعرضت إلى إصابة جراء البحث، أو لأي سؤال عن الإصابات المتعلقة بالبحث، يرجى الاتصال بالدكتور عمر عبيد
email: **oo01@aub.edu.lb**، 4440 مقسم 350000 (01)

ط) أسئلة ومعلومات الاتصال:
١) لأي أسئلة أو أي مخاوف حول البحث، يمكنك الاتصال بالدكتور عمر عبيد، قسم التغذية وعلم الطعام الجامعة الأمريكية في بيروت، شارع القاهرة، بيروت، لبنان
email: **oo01@aub.edu.lb**، 4440 مقسم 350000 (01)
٢) لأي أسئلة أو أي مخاوف حول حقك كمشارك في هذا البحث يمكنك الاتصال بالمكتب التالي في الجامعة الأمريكية في بيروت:مجلس المراجعة المؤسسية للعلوم الطبية
أو 5445 مقسم 350000 (01) الجامعة الأمريكية في بيروت، شارع القاهرة، بيروت، لبنان
email: irb@aub.edu.lb، 5440

ي) حقوق المشاركين: المشاركة في هذا البحث إختيارية. يمكنك مغادرة البحث في أي وقت من دون أي عقوبة. إن قرارك بعدم المشاركة لن يؤثر بأي شكل ممكن على علاقتك بالجامعة الأمريكية في بيروت و لن يؤدي الى أي خسارة شخصية. هل لديك أي أسئلة حول المعلومات الواردة أعلاه؟ هل ترغب في المشاركة في هذه الدراسة؟

الاتصال في المستقبل:

هل ترغب في الاتصال بك للمشاركة في أبحاث أخرى في المستقبل؟ نعم _____ لا _____
ملاحظة: للباحث الحق الكامل بإيقاف أي مشارك عن متابعة مشاركته في هذا البحث.
موافقة المشترك:
لقد قرأت استمارة القبول هذه وفهمت مضمونها. وبناء عليه فأنني، حرا مختارا، أجيز إجراء هذا البحث و أوافق على الإشتراك فيه .

إسم المشترك _____ التاريخ و الوقت _____ توقيع المشترك_____

الإسم المطبوع للشخص المأذون للموافقة من أجل الشخص:_____

العلاقة بالشخص: _____
إمضاء الشخص المأذون للموافقة:_____ التاريخ و الوقت:_____

توثيق الموافقة:

الإسم المطبوع للشخص الذي يطلب الموافقة:_____

إمضاء الشخص الذي يطلب الموافقة:_____
التاريخ و الوقت:_____

APPENDIX IV

ENGLISH CONSENT FORM

BIOMEDICAL NUTRITION CONSENT
AUB

Title of Research Study: The relation between adiposity, inflammation, glycaemia and iron absorption: a comparison between central and peripheral adiposity.

Principal Investigator: Dr. Omar Obeid/ Faculty of Agricultural and Food Sciences/ Department of Nutrition and Food Science/ American University of Beirut
Co-Investigator: Dr. Bassem Safadi, Dr Marwan Refaat, Dr. Hala Ghattas, Dr. Imad Toufeili
Researchers: Carla Al Mallah
Address: American University Beirut, Cairo Street, Hamra, Beirut – Lebanon/01 – 350 000
Site where the study will be conducted: American University of Beirut- Department of Nutrition, Faculty of Agricultural and Food Sciences; Central Research Unit (CRU), American University Beirut, Medical Center: Medical centers of Rafik Hariri Foundation.

We are asking you to participate in a research study. Before agreeing to participate in the research, it is important that you read the information below. This statement describes the purpose, procedures, benefits, risks, discomforts, and precautions of the study. Also described are the alternative procedures, if any, available to you, as well as your right to withdraw from the study at any time. You should feel free to ask any questions that you may have.

A. Purpose of the Research Study: Low iron status has been reported among overweight and obese subjects for a long time. Several etiologies exist, some of which are related to the intake of iron, while others have linked it to the type of foods consumed by the population. However, further investigations have shown that this reduced level of iron is not related to the actual intake of the mineral but rather to its rate of absorption that is affected by the adiposity level. However, iron supplementation is commonly prescribed to low iron status overweight and obese subjects regardless of their capacity to absorb iron. Additionally, these individuals happen to have a chronic low grade inflammatory status that has been witnessed to decrease iron absorption, creating a state similar to that of anemia of chronic diseases (ACD). The aim of this study is to investigate the effects of the two most common types of adiposity (central vs. peripheral) on iron absorption, and whether the glycemic and inflammatory profiles of the individual have any effect on its absorption rates, and how they might be implicated in the development of iron deficiency in these individuals.

B. Recruitment of Participants: The recruitment process in this study will involve contacting physicians at the American University of Beirut Medical Center, other hospitals and health and dietetic centers in Lebanon, in addition to personal contacts. After obtaining the approval of physicians, investigators will approach potential participants in the waiting area at the clinics, whereby the study will be explained to them, and hence, those who are interested and fit the inclusion criteria will be asked to sign a consent form and they will fill a questionnaire about their socio-economic, demographic and health status.

C. Project/Procedures Description: In this study, you will be asked to maintain your regular dietary and physical activity habits during the entire study course, avoid alcohol consumption as well as any unusual strenuous exercise 24 hours prior to the study. Exclusion criteria include: individuals suffering from diseases that may be affecting their blood, such as thalassemia, in addition to the presence of any inflammatory diseases, and subjects who are on iron supplementation.

The only preparation you need to do on your own is to come fasting for the last 12 hours and stop the ingestion of any nutritional supplement.

As a first step, you will visit the department of Nutrition and Food Sciences at AUB, where anthropometric measurements (height, weight, WC), in addition to a body composition analysis using two methods: bioelectrical impedance analysis (BIA) where the individual will stand on a digital scale which runs a current through the body in order to determine its composition (bones, fat, muscles, water, and their specific distributions) and the dual energy X-ray (DEXA) where the individual lays down without moving on a scanning bed and the body will be scanned using X-rays in order to determine its composition (fat, muscle, water, bones… and their specific distributions), and you will be asked to fill questionnaires about your health and socio-economic status. Moreover, in order to ensure the status of the woman, a pregnancy test will be conducted in order to make sure that there is no current pregnancy to avoid any unforeseen risks. This step is done in order to be classified in one of the three groups of interest (lean, central obesity, peripheral obesity). If you fit the inclusion criteria, then you will be asked to attend one of the experimental sites after a 12 hours overnight fast. A blood sample will be drawn in the fasted state. Following that, you will be given a test meal (bread with butter and honey) containing 6 mg of stable labelled iron isotope ^{57}Fe. You will be asked not to eat or drink for 3 hours after the ingestion of the meal to avoid the effect of any external influencing factors on the iron absorption. Finally, you will be asked to show up after exactly 14 days in a fasted state at the same experimental site for a second blood withdrawal. Following that, you will be given an OGTT solution (50 grams dextrose) which includes 100 mg of iron in the form of sodium ferrous citrate (SFC). A third blood sample will be drawn 2 hours after the ingestion of the oral mixture.

The study will be fully covered and the individuals are not requested to pay anything in order to participate. Half of the study will be covered by ETH Zurich.

D. Duration: The estimated time to complete this experiment is 2 weeks. You will have to visit the testing facility 3 times. You will have to stay for around 30 minutes during your first visit at the department of Nutrition at AUB to finish the assessment. The second visit, which will be at one of the experimental sites, will last for about one hour. As for the last visit, it will require between 2 to 3 hours.

You may leave the study at any time. If you decide to stop participating, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled. Your decision will not affect your future relationship with AUB.

E. Risks, Discomforts and Benefits: Your participation in this study involves only minimal risks. You will rest comfortably in a chair. An intravenous catheter (small plastic tube) will be placed in a vein of your forearm by a qualified nurse for blood collection. There are no risks involved in this procedure. In addition, the DEXA scan is very easy and doesn't cause any side effects as far as we know. Although there are no proven harmful effects from the radiation levels that you will be exposed to during this research, long term effects on your health cannot be ruled out with certainty. You may not participate in this study if you are pregnant. If you are capable of becoming pregnant, a pregnancy test will be performed before you are exposed to any radiation. You must tell us if you may have become pregnant within the previous 14 days. You will be sked about the first day of your last menstrual period and the DEXA scan will be performed to you only within 10 days interval after the first day of your last menstrual period.

The iron supplements taken at large doses usually cause some gastric discomfort raging from nausea to bloating. However, in our study, the quantity and type of iron used are safe and cause no health risks according to our knowledge.

The foreseeable risks and discomforts associated with the study are as follows:

- The indwelling catheter for blood samples may cause a range of risks: from simple redness of the skin to a local inflammation of the vein, and some rare incidents of fainting and hematoma formation may occur.

The following measures will be taken to reduce the above mentioned risk:

- Before we start, the procedure and measurement techniques will be reviewed with you to ensure that you are comfortable with the protocol.
- All tests will be carried as per the standard clinical procedures.
- To ensure that there are no risks associated with the use of indwelling catheters, the skin area will first be sterilized with alcohol. A qualified nurse will place the catheter, ensure its correct operation and collect the blood samples. All blood samples will be taken with sterilized instruments. The nurse will also withdraw the catheter at the end of the experiment. Once the catheter is removed, the wound is cleaned with alcohol and peroxide. A sterile bandage is then applied. You will be asked to report any unusual discomfort or discoloration of the skin.
- The use of a catheter will provide less discomfort compared to repeated sampling with a needle. However, there may be some pain felt upon the insertion of the catheter, but no discomfort will occur during blood withdrawal.

The procedure followed in the study will not cause any major risk other than discomfort from the needle prick for blood withdrawal as mentioned in the procedures above. However, there may be unforeseen risks.

Your participation in this study provides you with no personal benefits, however, you will be helping in the determination of new diagnostic criteria and guidelines for iron deficiency in cases of obesity and overweight, and thus help in reducing the prescription of iron supplement to all women with elevated BMI.

F. Confidentiality: To secure the confidentiality of your responses, your name and other identifiers will never be attached to your answers. All codes and data will be kept in a locked drawer in a locker room or in a password protected computer that is kept secure. Data access is limited to the Principal investigator and researchers working directly on the project. All data will be destroyed responsibly after the required retention period. Your privacy will be maintained in all published and written data resulting from this study. Your name or other identifying information will not be used in our reports or published papers.

There may be circumstances where your confidential information must be released. For example, personal information regarding your participation may be disclosed if required by the AUB IRB, the U.S. Office of Human Research Protections or other federal or international regulatory agencies, or the sponsor of the study, if any, or agency supporting the study.

G. Compensation/Incentive: You will be receiving an amount of $50, divided over the two visits, for your contribution in the study in order to cover for the transportation to and from the research center unit. You will also receive the results of your blood tests conducted during the different phases of the study, as well as the body composition results including percent body fat, muscle mass, and total water.

H. Payment for Research-related Injury: In case of any adverse event, AUBMC will cover the cost of treating, on its premises, medical adverse events resulting directly from the medication and/or medical procedures of this research study. Otherwise, it will not cover for the costs of medical care for any medical condition or issue.

If you are injured as result of participating in this study or for questions about a study-related injury, you may contact Dr. Omar Obeid at 01/355555-ext 4440 or send him an email at oo01@aub.edu.lb.

I. Contact Information and Questions:
1) If you have any questions or concerns about the research you may contact:
Dr. Omar Obeid, 01/355555-ext 4440; oo01@aub.edu.lb.

2) If you have any questions, concerns or complaints about your rights as a participant in this research, you can contact the following office at AUB:
Biomedical Institutional Review Board: irb@aub.edu.lb, 00961 1 350000-ext 5440 or 5445

J. Participant Rights: Participation in this study is voluntary. You are free to leave the study at any time without penalty. Your decision not to participate in the study does not influence your relationship with AUB, and any refusal to participate will have no loss of benefits Do you have any questions about the above information? Do you wish to participate in this study?

K. Future Contact
Would you like to be contacted for future research? Yes _____ No _____
Please notify that the investigator has the right to end subject's participation in this study.

Participant Consent:
I have read and understand the above information. I agree to participate in the research study.

Participant Name: _____ Date & time: _____

Participant Signature: _____

Printed Name of person authorized to consent for subject: _____

Relationship to Subject: _____

Signature of Person authorized to consent: _____ Date & time: _____

Documentation of Consent:
Printed Name of Person obtaining Consent: _____

Signature of Person obtaining Consent: _____
Date & time: _____

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