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The Involvement Of Obesity In Changes Of Hepcidin And Iron Status In Najaf - Iraq

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ABSTRACT

Obesity have become a global problem affects both, high income countries and developing countries. It is considered as a low grade inflammation state associated with excessive production of cytokines. Hepcidin is a polypeptide hormone, negatively controls serum iron metabolism. It is up regulated in response to inflammation and is thought to play a role in the manifestation of iron deficiency (ID) observed in obese populations. The involvement of hepcidin in changes of iron state and development of iron deficiency anemia in obese Iraqi individuals is not clear There were significant decreases in concentrations of hemoglobin (P<0.01), iron (P<0.0001) and Tsat% in obese men when compared with those of the control group. On the other hand, significant increases in levels of hepcidin (P<0.0001), ferritin (P<0.0001), TfR (P<0.0001), CRP (P<0.0001), EPO (P<0.001) and TIBC (P<0.001), were obtained in the group of obese men with respect to those of the control group. The linear regression analysis exhibited significant positive correlations of hepcidin levels with values of body mass index (r=0.52, P<0.001), TfR (r=0.42, P<0.001), EPO (r=0.24, P<0.05) and CRP(r=0.31, P<0.05) in the group of obese men but not in the control group. However hepcidin concentrations were found to be significantly (r = - 0.40, P<0.001) negatively correlated with iron levels in the group of obese men and decline in Tsat % (r = 0.14, p< 0.05. A hematological examination confirmed the diagnosis of iron deficiency anemia in 35 (38%) out of the 95 investigated men.

Keywords: Obesity, Hepcidin, Iron status (iron deficiency).

INTRODUCTION

Obesity is a medical disorder in which excessive accumulation of body fat may cause serious health problems. Obesity increases the risk of type 2 diabetes, hypertension, heart disease, stroke, dyslipidemia, osteoarthritis, gynecological problems, sleep apnea and respiratory problems (Reijman et al., 2007) [1]. It is well documented that obese people are more susceptible to suffer some types of cancer, infections and a prolonged time of wound healing after surgery (Olsen et al., 2007;[2] Guo and Dipietro, 2010) [3]. Obesity in adults is measured in terms of a person's body mass index (BMI) which is determined both by weight and height. For adults, the cut-off points used are: BMI < 18.5 indicates that a person is underweight; BMI 18.5-24.9 is the desirable or healthy range; BMI 25- 29.9 is classified as overweight; and BMI more than 30 is classified as obese. Hepcidin is a recently identified peptide hormone. Human hepcidin gene (HAMP) is located on human chromosome 19q13 and encodes a precursor protein consisting of 84 amino

acids. Hepcidin is released into the blood circulation as a peptide containing 20, 22 or 25 amino acids. This protein is synthesized mainly in the liver, but also in the heart, pancreas, kidney, spleen and adipose tissue (Leong and Lönnerdal, 2004) [4].

Hepcidin is a negative regulator of iron metabolism. It inhibits the absorption of iron in the small intestine and the release of recycled iron from macrophages. Thus, hepcidin affects the amount of iron available for erythropoiesis. There are many different factors that regulate hepcidin gene expression. The synthesis of hepcidin in the liver is increased by inflammation, infection and transferrin saturation. In contrast, hypoxia, erythropietic activity and mutations in the hepcidin gene (observed in hemochromatosis) decrease its expression (Starzyński, 2004[5]; Ganz and Nemeth, 2006) [6] . Interestingly, hepcidin concentrations in the hepatic and adipose tissues correlate with different iron status markers and inflammation indexes. The regulation of hepcidin gene expression and protein synthesis is probably tissue specific. These data appear to strengthen the hypothesis that obesity, as a low grade inflammation state, stimulates the production of many cytokines and adipokines such as IL-6 and leptin. Leptin can up-regulate hepcidin synthesis by adipocytes. As a result, increased hepcidin levels may lead to poor iron status in obesity, by inhibiting iron absorption and restricting iron bio-availability. Most studies have shown similar results. A cross sectional study by Phinas-Hamiel and coworkers (2003) [7], described a greater prevalence of iron deficiency (ID) in overweight and obese. In this study, ID was defined as iron concentration < 8 μmol L^{-1}, and iron deficiency anemia (IDA) was defined as a hemoglobin level below 2 standard deviations (SDS) for age and gender. Subsequently, a large study confirmed those findings where it was demonstrated that overweight were twice as likely to be iron deficient as normal weight human (Nead et al., 2004) [8]. The high BMI group had significantly lower serum iron levels and markedly higher C-reactive protein (CRP) and soluble transferrin receptor levels (sTfR).

Ferritin is present in blood in very low concentration. Normally approximately 1% of plasma iron is contained in ferritin. The plasma ferritin, is in equilibrium with body stores, and variations of iron storage. The serum or plasma concentration of ferritin decline very early in anemic conditions like development of iron deficiency, long before the changes are observed in the blood hemoglobin concentration, size of the erythrocytes and total iron binding capacity (TIBC).

Erythropoietin (EPO) is a heavily glycosylated protein with a molecular weight of about 30 – 34 Kilo Daltons. Human EPO is a polypeptide consisting of 165 amino acid, containing one O- linked and three N-linked carbohydrate chains[9] . Serum EPO levels are dependent on the rate of production and the rate of clearance of the protein. Ninety percent of EPO is produced in the peritubular cells of the adult kidney in response to a decrease in tissue oxygenation [10]. The aim of this study was designed to investigation and evaluate the role of hepcidin in directing changes of the iron state and development of iron deficiency anemia in obese Iraqi men.

MATERIALS AND METHODS

Patient and control subjects: Ninety five obese men {body mass index (BMI) ≥30.0 Kg/m^2} and forty non-obese (lean) men (BMI:18 – 24.6 Kg m^2) as controls groups were recruited from Medicine Collage - University of Kufa participants attended the biochemical laboratory. Blood samples were taken from individual (men) obese and non obese (lean) as control group withdrawn in tubs by disposable syringes and needles were used for blood collection. Blood samples were obtained from obese and non obese controls group by vein puncture and divided 1 ml in EDTA tub and the remain sample were allowed to clot at 37 ^{0}C then centrifuged at 3000 Xg for 10 minutes and prepared serum rapidly and stored at -20 ^{0}C until analysis. Biochemical data are presented as (mean ± SD). Compressions were assessed using paired t – test, and differences between groups were assessed using Students t- test for continuous variables and exact test for categorical variables.

Method: Hemoglobin was measured by colorimetric test (Cypress diagnosis)[11]. Serum iron, total iron binding capacity (TIBC), transferrin saturated (Tsat%) and C-RP, serum ferritin, erythropoietin(EPO) and transferrin receptor (sTfR) were performed respectively by chemical laboratory of biochemistry in medicine college of Kufa university in Najaf - Iraq.

Serum iron and TIBC were measured by (CAB- method) (Human Gesellschaft for biochemical and diagnosis. Germany) [12,13]. Serum iron reference value 59 – 158 µg dl^{-1} or (µmol L^{-1}) for males and 37 - 145 µg dl^{-1} for females and TIBC reference value 274 – 385 µg dl^{-1} and calculated UIBC = TIBC – Iron. Reference value 180 – 260 µg dl^{-1} .Ferritin was measured by AccU - bindTM Micro plates procedure [14]. Reference value 16 – 220 ng ml^{-1} for males and 10 – 124 ng ml^{-1} for female's consistence with iron deficiency (ID) based on laboratory outpoints. (Tsat)% was calculated as Iron/ TIBC x100 [15]. Erythropoietin (EPO) was measured by enzyme immunoassay for quantitative determination erythropoietin (EPO) in human serum (Demeditec diagnostic Gmb. Lis – Meitner- Straße. Germany)[16]. With an expected reference value 13.8 – 21.9 mIU ml^{-1}. Serum transferrin receptor (sTfR) was measured by Quantikine IVD immunoassay (R&D system Minncaplis,MN), The manufacturer ,s expected reference value for this assay is 8.7 – 28.1 nmol L^{-1} with a value > 28.1 nmol L^{-1} indicative of ID per manufacturer's recommendations. Hepcidin was assayed by immunoassay kit allows for the invitro quantitative determination of human Hepcidin concentration in serum (Human Hepcidin ElISA kit CUSABIO) with an expected reference value of 12.5 – 400 ng ml^{-1}. C-reactive protein (CRP) was measured by immunoturbidity (reference interval < 1.0 mg L^{-1}).

Biostatistics analysis : The result were expressed as mean ± SD. Students t-test was used for comparison of results of obese and non obese (lean) as control group. Significant variation was considered when p - value was less than < 0.05. The correlate between the values of hepcidin, body mass index (BMI), iron status parameters, C-reactive protein(CRP) and various factor were performed by the liner regression analysis (Table 2) and histogram figure (1.a) obese and figure (1.b) non obese.

RESULTS AND DISCUSSION

Ninety five men were recruited obese and forty men non obese (lean) as control groups. In the current study we observed non significant (NS) between the study obese and non - obese control groups for age (Table 1). Body mass index (BMI) was significantly higher in obese (38±1.7) compared to non-obese control (22.9±1.6); $p < 0.001$, respectively (Table 1). Hb (10.5 ± 1.6), serum iron (45±6.1) and transferrin saturation (7.6±2.3) were all significantly lower in obese men compared to the non-obese (lean) controls group (14±1.5) (138±23.2), (42±14.1) $p<0.0001$, respectively. All hepcidin (Hep)(480 ±150) $p<0.0001$, TIBC(575±72.8)$p<0.001$, UIBC(530±66.7) $p<0.0001$, transferrin receptor (150±19.8) $p<0.0001$, ferritin(288±12.4) $p<0.0001$, Erythropoitin (EPO)(27±5.9) $p<0.001$ and C-reactive protein(C-RP)(11±1.5) $p<0.0001$ were significantly higher in obese when compared to the non-obese control group respectively (Table 1) (Fig 1 a, b).

Correlation between hepcidin and clinics-laboratory parameters in obese such as serum hepcidin showed significant Positive correlation with BMI ($r=0.525$, $p<0.001$). On the contrary serum hepcidin in obese showed negative correlations with Hb, serum iron($r= -0.51$,$p<0.001$) and transferrin saturation (NS)($P > 0.05$) (Table 2). Otherwise it showed significant positive correlations with TIBC, UIBC, TfR and ferritin ($P < 0.001$), (Table2). Also serum hepcidin in obese was positive correlation with erythropoietin and slightly elevated with C- reactive protein (Table 2).

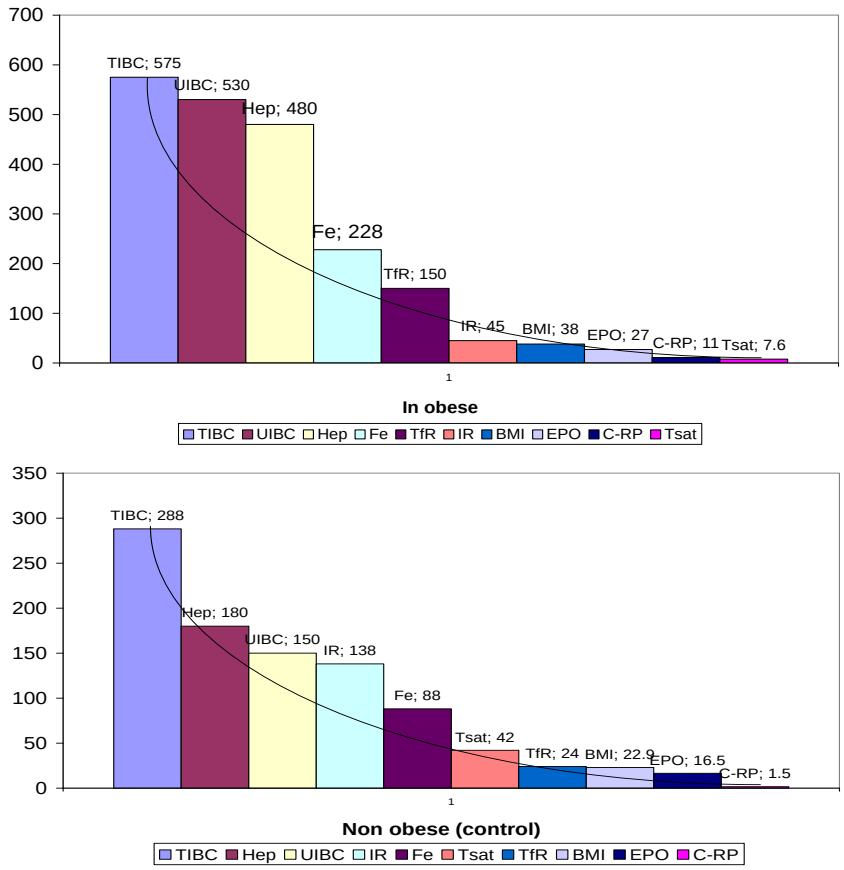


Fig 1.a,b Histogram showing level of total iron binding capacity (TIBC), unsaturated iron binding capacity(UIBC), hepcidin(Hep), iron(IR), Ferritin(Fe),transferrin saturation(Tsat %), Body mass index(BMI), Transferrin receptor(TfR), erythropoitin(EPO) and C- reactive protein (C- RP).

Table 1. Clinical and laboratory data of patients obese and non-obese (control group).

Patients Obese n = 95			Controls Non- Obese n = 40		
Parameter	Mean ± SD	Range	Mean ± SD	Range	P - value
Age years	37.5± 9.4	25 – 50	35.5 ± 8.2	25 – 46	NS
BMI kg/m2	38 ± 1.7	30 – 39.3	22.9 ± 1.6	17.8 – 26	<0.001
Hb g/dl	10 .5± 1.6	10.2-12.5	14 ± 1.5	13 – 15	<0.01
Hepcidin ng/ml	480 ± 150	183 – 630	180 ± 38.5	38.5 – 208	<0.0001
TIBC µg/dl	575 ± 72.8	150 – 630	288 ± 36.4	85.5 – 350.5	< 0.001
UIBC µg/dl	530 ± 66.7	95 – 600	150 ± 13.2	110 – 235	<0.0001
TfR nmol/l	150 ± 19.8	33 – 184	24 ± 2.9	10 – 48	<0.0001
Ferritin ng/ml	228 ± 12.4	38.8 – 240	88 ± 15.6	26.8 – 104.9	< 0.0001
IRON µg/dl	45 ± 6.1	35 – 53	138 ± 23.2	95 – 170	<0.0001
EPO mIU/ml	27 ± 5.9	16.6 –33.9	16.5 ± 5.25	16.5 – 23.2	<0.001
Tsat%	7.6± 2.3	6.4- 12.6	42 ± 14.1	42 – 70.5	<0.0001
C-RP mg/dl	11± 1.14	4.6 – 12	1.5 ± 0.3	0.8 – 2	<0.0001

Table 2. Correlation of serum hepcidin with select anthropometric, biochemical variables analyzed in all subjects.

	Obese		Non-obese (control)	
Parameter	r	P - value	r	P – value
Hep with BMI	0.525	<0.001	0.2	NS
Hep with Fe	0.4	<0.005	0.144	NS
Hep with Iron	- 0.51	<0.001	0.48	<0.001
Hep with EPO	0.238	<0.05	0.16	NS
Hep with Tsat%	0.14	NS	0.39	<0.05
Hep with TfR	0.42	<0.001	0.15	NS
Hep with C-RP	0.32	<0.05	0.13	NS

Obesity have significantly higher serum hepcidin levels compared to non-obese (leans) as control group with serum parameters of iron status ferritin, TIBC, EPO, TfR) (Table 1,Figer 1a,b) and significantly low Hb, serum iron and Tsat% . Further, those obese individual suffer from a functional iron deficiency(ID) as indicated by elevated transferrin receptor (sTfR) and mostly undetectable iron in their abdominal adipose and liver macrophages[17]. Obesity-associated increase in hepcidin, via the JAK/ STAT3 pathway, and consequent reduction in absorption of dietary iron is thought to partially compensate for the defect in the Hfe gene resulting in less severe iron loading in the obese patients [18]. In further support, a study in obese women and children with suboptimal iron status and elevated transferrin receptor (sTfR) reported decreased dietary absorption of iron-fortified foods compared to non-obese (lean) controls and confirms that iron absorption is diminished in obese individuals despite apparent functional iron depletion [19](Figure 1).

Data from the American National Health and Nutrition Examination Survey III as well as data obtained in individual from transition countries (Morocco and India) have suggested that among adults individual, the prevalence of iron deficiency increases as BMI increases from normal weight to at risk for obesity to obesity [19,20,]. It remains unclear, however, if the lower serum iron and elevated ferritin seen in obesity are most reflective of a functional iron deficiency related to an inflammatory state, or if obesity is also a risk factor for true iron deficiency [21]. At the beginning of our study, obese individual with iron deficiency (ID) had significantly higher serum ferritin levels than non-obese (lean) control group ($p < 0.01$) (Table 1). Serum ferritin concentrations, which are usually suppressed when body iron stores are low [22], tend to be high and inversely related to transferrin saturation in those with excessive adiposity. Ferritin is considered an acute-phase reactant [21], hence, it may be elevated in inflammatory conditions even in the presence of true iron deficiency [22]. Cytokines such as interleukin-1β and tumor necrosis factor-α (TNF-α) induce ferritin production within macrophages, hepatocytes and adipocytes [23].

In our study, obese individual with ID had significantly higher serum hepcidin levels ($p < 0.0001$), in comparison to non-obese control group in contrast, non-obese individual control had significantly lower serum hepcidin levels, compared to obese $P< 0.0001$ (Table 1). Hepcidin is suppressed in iron deficiency, allowing increased absorption of dietary iron and replenishment of iron stores [24]. The feedback loop between iron and hepcidin ensures stability of plasma iron concentrations[25]. Hepcidin is an acute-phase reactant [21], and its expression is increased in chronic inflammatory states[26] including obesity [27]. Hepcidin can inhibit enterocyte iron absorption [28] and has further been shown to inhibit the release of non-hems iron from macrophages [29]. Because each of these actions diminishes the amount of bioavailability body iron, it has been suggested that when hepcidin is induced by inflammation, hepcidin is a key iron regulator that causes the hypoferremia and anemia of chronic disease [30].

In the present study, C-reactive protein (C-RP) was significantly higher in obese individual with ID than in non-obese control group ($P < 0.001$, respectively). Yanoff et al [21] found that C-RP concentrations were

higher in obese subjects and were positively correlated with BMI, findings consistent with the observation that obesity is an inflammatory state that increases acute-phase reactants. Zimmermann et al [19] stated that adiposity in young women predicted not only lower iron absorption but also reduced response to iron supplementation, possibly due to increased hepcidin production. Serum hepcidin in non-obese, showed significant positive correlation with Hb, serum iron and transferrin saturation ($P < 0.01$). In contrast, in obese individual, serum hepcidin showed significant negative correlation with Hb, serum iron and transferrin saturation ($P < 0.05$) (Figer 2 a,b).

Erythropoietin(EPO), quantization of serum erythropoietin concentration serves as a diagnosis adjunct in determining the cause of anemia or erythrocytosis. A plastic anemia, and anemia due to iron deficiency all result in serum elevation(EPO). An increased concentration of EPO verifies that anemia is due to red cell hypoplasia or aplasia(31). Later studies have utilized and similar to the Lecube et al(32) and Menzie et al.(33) studies, this study uncovered significant correlations between serum iron, sTfR, fat mass, and BMI in adults. Collectively, these reports suggest that excess adiposity may negatively affect iron status. As a result, increased hepcidin levels may lead to poor iron status in obesity, by inhibiting iron absorption and restricting iron bioavailability.

Aeberli and coworkers (2009)(34) also studied hepcidin concentration in parallel with iron intake, iron bioavailability, serum ferritin, sTfR, C-RP and leptin in a group of 6-14 year old overweight children, compared with normal weight children. The prevalence of iron deficiency among overweight subjects was significantly higher than in normal weight controls (20% vs 6%). Inflammatory markers (IL-6 and C-RP) as well as hepcidin and leptin were significantly elevated in obese children. The authors of this study concluded that iron deficiency in obese children was caused by a hepcidin-mediated reduced iron absorption and/or an increased iron sequestration. Richardson and coworkers (2009)(35) conducted a prospective study to answer the question whether low iron status described in obesity is associated with the inflammation process. They evaluated high sensitivity (C-RP), iron metabolism parameters (serum iron, ferritin, transferrin saturation) and weight status (BMI).. Iron deficiency in obese individuals may be a result of low iron intake (e.g. due to an unbalanced diet), reduced iron absorption in the small intestine, and greater iron requirements caused by a larger blood volume. In addition, obesity is associated with a chronic low-grade inflammation state. For this reason, sequestration of iron through an inflammatory mediated mechanism can be one of the proposed causes of iron deficiency in obesity (Yanoff et al., 2007(36); Menzie et al., 2008)[33].

Bekri et al. [12,37]. Feedback regulation to iron via holo-transferrin exists in the liver but similar regulation may not exist in other tissues, such as the adipocyte [38]. It has been suggested that the exaggerated fat mass present in obesity could contribute significantly to systemic hepcidin concentrations or it may have a localized effect (i.e., iron sequestration in adipose-associated macrophages) [37]. Both hepcidin and ferritin are correlated in many conditions (iron-replete healthy individuals, ID anemia, and inflammation) and are regulated by the same stimuli; inflammation and iron [24,39]. Therefore, it was not surprising that both were highly correlated in the obese and non-obese individual. Of surprise was the apparent lack of correlation between serum hepcidin and C-RP in the obese individuals. (Finally, we can conclude that obesity increased hepcidin levels and was associated with diminished iron deficiency. The results are in good agreement with other studies [33] and Richardson&Coworker2009)[35].

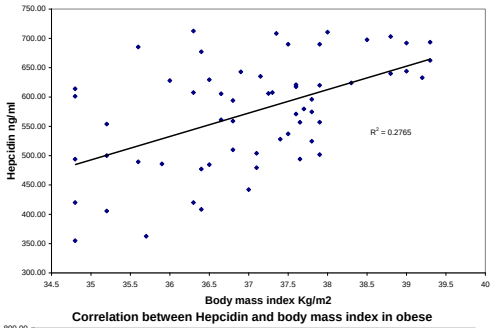
APPLICATIONS

The current investigation is designed to evaluate the role of hepcidin in directing changes of the iron state and development of iron deficiency anemia in obese Iraqi men.

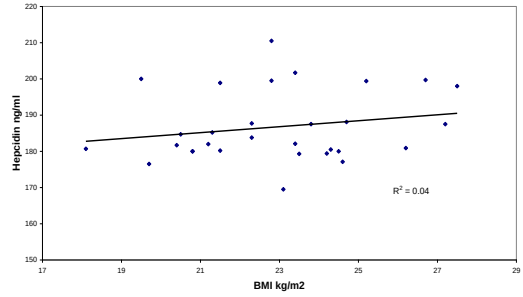
CONCLUSIONS

In conclusion, the present study supported the hypothesis that obesity in Iraqi men is associated with excessive secretion of hepcidin, resulting in iron deficiency (e.g. due to unbalanced diet), reduced iron absorption in the small intestine, iron storage and greater iron requirements by a larger blood volume in patients with obesity, a consequent iron deficiency anemia in approximately one third of these individuals. So obesity association with a low grade inflammation due to increased level of C-reactive protein(C-RP). In addition, increased levels of hepcidin and transferrin receptor more than reference value in obese indicate iron deficiency.

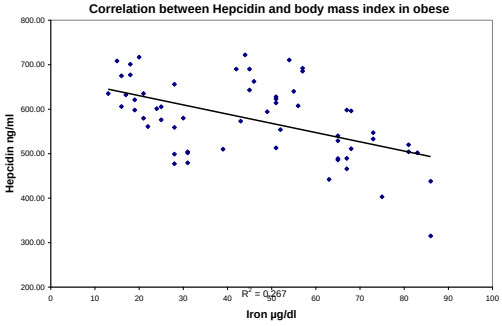
<u>Obese</u> <u>Non obese</u>



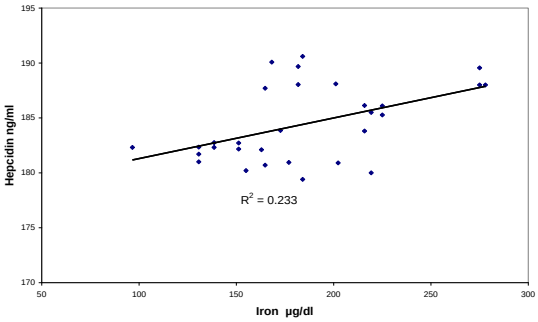
Correlation between Hepcidin and body mass index in obese



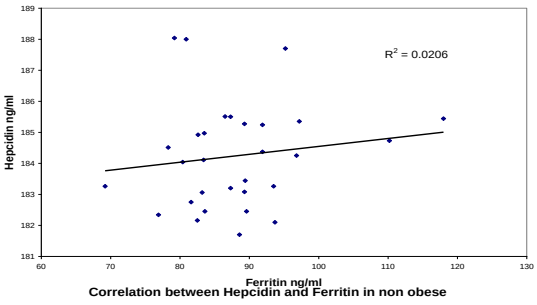
Correlation between Hepcidin and body mass index in non obese



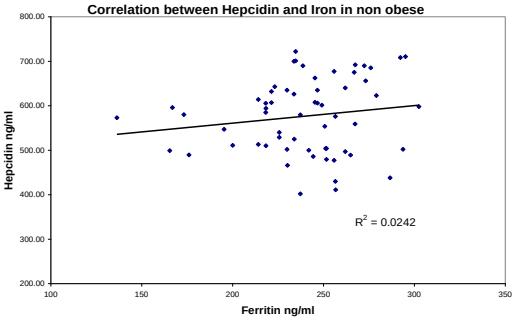
Correlation between Hepcidin and Iron in obese



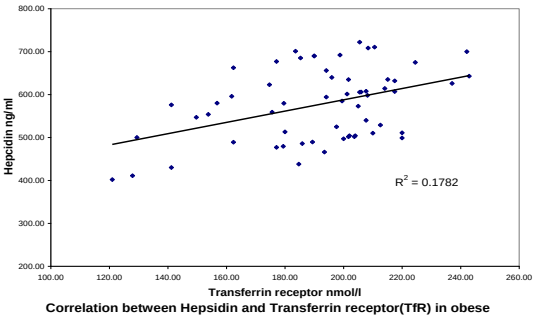
Correlation between Hepcidin and Iron in non obese



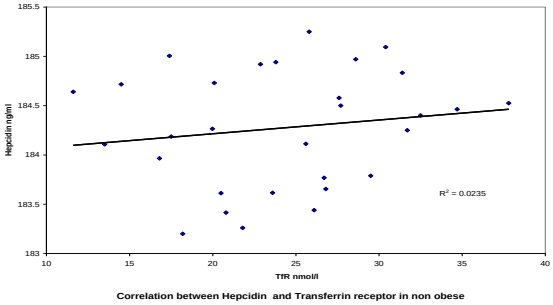
Correlation between Hepcidin and Ferritin in non obese



Correlation between Hepcidin and Ferritin in obese



Correlation between Hepsidin and Transferrin receptor(TfR) in obese



Correlation between Hepcidin and Transferrin receptor in non obese

Selective correlation between Hepcidin and Iron **status**

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