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A systematic review on serum retinol binding protein 4 (RBP4) and interrelated iron status in type 2 diabetes mellitus patients

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***Abstract*---**The dysregulation of adipokines is closely associated with the pathogenesis of insulin resistance and type 2 diabetes mellitus. A recently proposed adipokine i.e., Retinol-binding protein-4 (RBP4), is found to provide a connecting link between insulin resistance and obesity. Interestingly, the interaction between vitamin A and iron status has also been observed in a few shreds of evidence. For instance, vitamin A deficiency may result in impairment of iron metabolism and may aggravate anemia. Here, the present review is designed to summarize the up-to-date knowledge on RBP4's role in obesity, development of insulin resistance, and Type 2 Diabetes Mellitus by findings of previous studies and investigate the crucial possible outcome of the relationship between RBP4 and iron status in type 2 diabetes mellitus patients, most importantly in humans.

***Keywords*---**Adipokines, RBP4, Insulin Resistance, Iron Status.

Introduction

As we know, adipose tissue is well-known as an endocrine organ that secretes a number of adipokines (such as tumor necrosis factor-α, leptin, interleukin 6, and adiponectin) that control insulin activity in other tissues.[1] Retinol Binding Protein 4, a newly discovered adipokine, has recently been associated with type 2 diabetes mellitus and has been proposed as a link between inadequate glucose absorption in adipocytes and systemic insulin sensitivity in several diabetic mouse models.[2] In humans, increased levels of serum RBP4 have been observed by several authors in obese, insulin-resistant, or type 2 diabetes mellitus populations.[3,4]

Experimental studies have suggested the injection of purified RBP4 into mice was shown to induce hepatic expression of phosphoenolpyruvate kinase in addition to impaired muscle insulin signaling, indicating the potential role of RBP4 in the development of type 2 diabetes mellitus. Furthermore, genetic knockout of GLUT4 expression in adipose tissue of mice results in reciprocal changes in adipose RBP4 expression and circulating levels of RBP4.[5] However, the exact mechanism by which RBP4 results in insulin resistance in humans is still under discussion.
In another aspect, diet and lifestyle play a major role in the prevention of type 2 diabetes mellitus. Iron is one of the most important micronutrients for our body and an altered iron profile may cause damage to many cellular structures caused by oxidative damage produced by free radicals, resulting in many complications associated with diabetes mellitus.

Elevated iron stores may induce diabetes through a variety of mechanisms, including oxidative damage produced by free radicals to pancreatic beta cells, impairment of hepatic insulin extraction by the liver, and interference with insulin's ability to suppress hepatic glucose production.[6]
Since iron affects the metabolism of glucose and glucose metabolism impinges on several iron metabolic pathways, the relationship between them is bidirectional. These relationships are influenced by oxidative stress and inflammatory cytokines which may amplify and potentiates the initiated events. Recent studies have shown an increase in iron stores (Ferritin) predicts the risk of developing type 2 diabetes, while a decrease in iron level is protective.[7] Hence, the Iron profile must be considered in relation to type 2 diabetes mellitus.

Interestingly, RBP4 has long been used as a predictor of vitamin A (retinol) intake. The association between vitamin A and iron status has also been observed in a few shreds of evidence. For instance, it has been reported vitamin A deficiency may result in impairment of iron metabolism and may aggravate anemia.[8]

In past years as per studies, in the general population and in individuals with type 2 diabetes mellitus, increased iron intake and increased iron storage have been recognized as important, independent factors to insulin resistance. Iron supplementation, on the other hand, was dramatically found to enhance plasma retinol and RBP4 levels.[9]

Hence, RBP4 and iron stores in the body seem to affect insulin resistance. Based on the association between vitamin A and iron, it has been hypothesized that

raised iron reserves provide a link between elevated serum RBP4 concentration and insulin resistance.

Therefore, in view of the above statements, the present review was designed to summarize the up-to-date knowledge on RBP4's role in obesity, development of insulin resistance, and Type 2 Diabetes Mellitus. Special attention is given to studies evaluating various published studies or planning new studies on this exciting adipokine and investigating the crucial possible outcome of the relationship between RBP4 and iron status in type 2 diabetes mellitus patients most importantly in humans.

Retinol Binding Protein 4 (RBP4) – Discovery & Protein Structure

RBP4 belongs to the lipocalin family and is the retinol's specific transporter in the bloodstream. RBP4 has a molecular weight of 21 kDa and in humans, it is encoded by the RBP4 gene. It is produced primarily from the hepatocytes and adipocytes.[10]

Discovery

RBP4 was first described in 1968 by Kanai et al. and identified as a human plasma protein that is preferentially attached to retinol and operates as a retinol transporter in circulation. They further observe plasma from individuals who received radiolabelled retinol injections intravenously and purified the protein that had bound labelled retinol and termed it as Retinol binding protein (RBP). In addition to binding retinol, it was discovered to be circulating in conjunction with another, larger protein exhibiting prealbumin mobility on electrophoresis.[11] Since then, several studies on the biology of RBP4 have been published, delving into its structure, function, and potential role in relation to human diseases.

Structure

In humans, RBP4's main structure was determined to be a single polypeptide chain with 201 amino acids and three disulfide bridges. During protein processing, an 18-amino-acid N-terminal secretory signal peptide is cleaved. RBP4's full 3D structure was described by Newcomer et al. (1984). An N-terminal coil, a C-terminal α-helix with a coil region, and a characteristic β-barrel core, which was demonstrated as an eight-stranded up-and-down β-barrel, were illustrated using X-ray crystallography. RBP4 is made up of a single polypeptide chain that has a hydrophobic pocket where retinol can bind, (Figure 1). The RBP4-retinol complex then binds transthyretin (TTR) in blood circulation, preventing RBP4 from being filtered by the kidneys.[12]

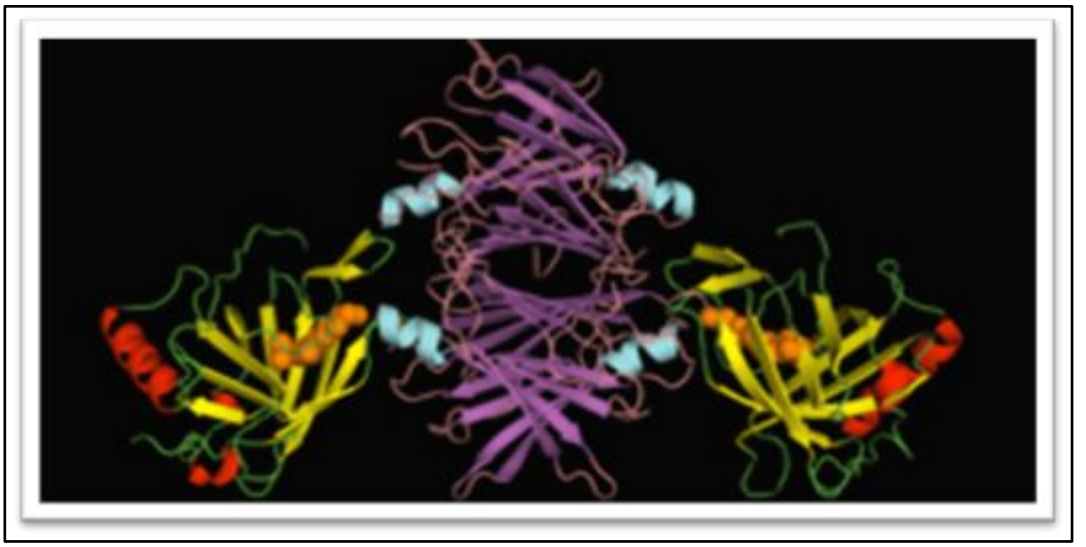

Figure 1: Two RBP4 molecules (yellow and red) are coupled to retinol (orange) and complexed with four TTR molecules (in purple and blue) (Naylor and Newcomer, 1999)

RBP4, Obesity, Insulin Resistance & Type 2 Diabetes Mellitus

Although elevated RBP4 levels in the blood of type 2 diabetic patients have been documented for many years, until, Yang et al., 2005 presented a causal relationship between circulating RBP4 and insulin resistance.[13] A mechanism whereby RBP4 modulates insulin sensitivity in muscle and liver has been suggested. In skeletal muscle, RBP4 reduces insulin sensitivity by inhibiting both insulin receptor substrate-1 phosphorylation and phosphatidylinositol 3-kinase activation, while increasing hepatic glucose production by increasing PEPCK expression, (figure 2).[14]

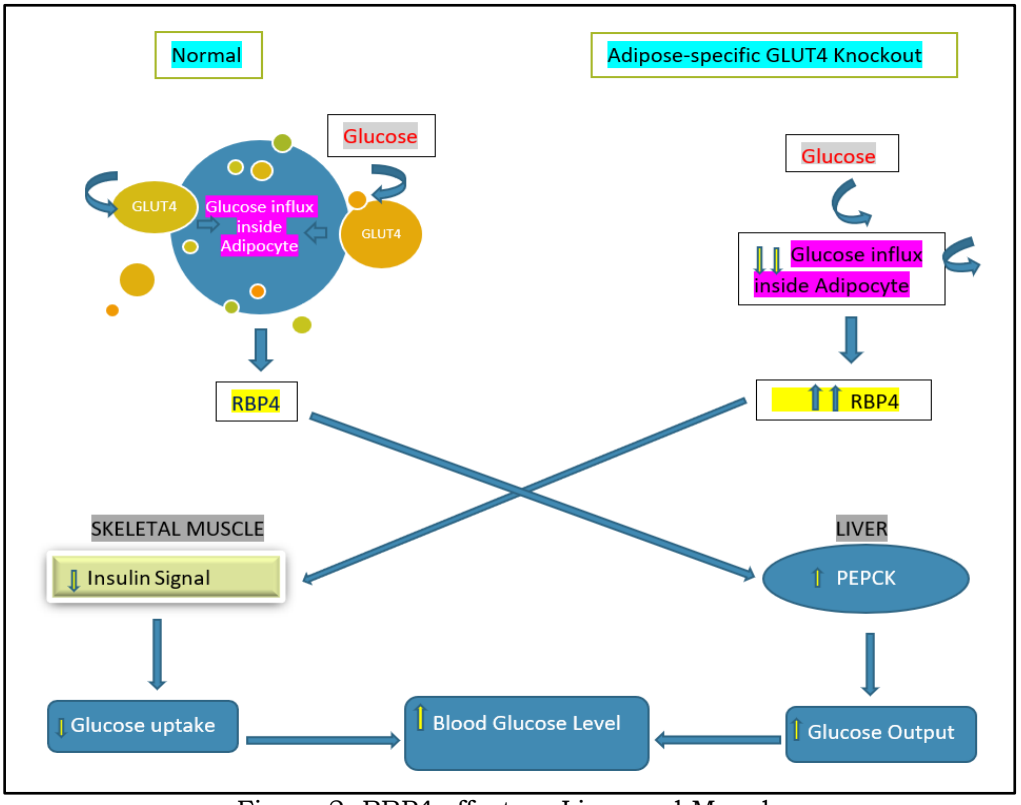

Figure 2: RBP4 effect on Liver and Muscle

Also, RBP4 was formerly thought to be a negative acute-phase inflammatory reactant. It has recently been suggested that RBP4-induced inflammation has been postulated as a possible cause of insulin resistance and cardiovascular risk. Fenretinide, a synthetic retinoid functions to increase the urinary RBP4 excretion, may reduce serum RBP4 levels and be found to improve insulin action in obese mice, thus this may have a therapeutic potential role in T2DM.[15] In humans, various researchers observed higher blood RBP4 concentrations in individuals with obesity, insulin resistance, or type 2 diabetes.[3,4] However, the exact mechanism is still under discussion.

Some noticeable contributions on RBP4 with type 2 diabetes mellitus are as follows

Name of first Author	Year	Study population/ Region	Study Type & Sample Size	Method for quantification	Statistical methods	Main findings
Timothy E Graham et al. [3]	2006	Group 1: 5 controls, 7 obese without diabetes, and 9 obese with Type 2 diabetes mellitus; Group	Case-control study, n = 107	-ELISA (ALPCO Diagnostics) in groups 1 and 3 and Western	-Linear correlations and nonparametric statistical tests were	In group 1, increased serum levels of RBP4 were found in obese individuals with/without Type

		2: 20 controls, 20 with impaired glucose tolerance, and 20 patients with T2DM; Group 3: 26 non-obese relatives of a Type 2 diabetes mellitus patient, USA, Germany, and Sweden		blotting in group 2, -Impaired glucose tolerance detected by an oral glucose tolerance test	performed with Analyse-it software (version 1.71), and independently confirmed with StatView (version 5.0, SAS Institute -Univariate Spearman rank correlation	2 diabetes mellitus In group 2, patients with impaired glucose tolerance and T2DM had greater serum RBP4 levels than those with normal glucose levels. In group 3, serum RBP4 showed no correlation with fasting plasma glucose but was positively correlated to fasting insulin (r=0.71)
Cho Y et al. [16]	2006	Subjects with normal glucose tolerance (NGT, n =57), impaired glucose tolerance (IGT; n=48) and type 2 diabetes mellitus (n= 49), Seoul, Korea	Case-control study, n = 154	Competitive ELISA, Impaired glucose tolerance is evaluated by an oral glucose tolerance test	ANOVA	Plasma RBP4 concentrations were higher in the IGT and type 2 diabetic groups than in the NGT group (median 18.9 [range 11.2–45.8], 20.9 [9.9–48.5], and 18.1 μg/ml [9.3–30.5], respectively).
Takebayshi K et al. [17]	2007	101 type 2 diabetic patients (62 men and 39 women) & 22 healthy controls, In 22 non-hospitalized type 2 diabetic patients, pioglitazone (30 mg/d) was administered for 12 weeks while other medications for diabetes were continued, Japan	cross-sectional study, n = 101	EIA kit (Phoenix Pharmaceuticals, Inc., Burlingame, CA).	-Simple linear regression for RBP4. -Paired t-test for comparison	A significant elevation of RBP4 levels in the 101 patients with type 2 diabetes (23.8±8.8 μg/ml), compared with the levels in 22 controls without retinopathy subjects (21.1±4.3 μg/ml), (P= 0.0352). No effect on serum RBP4 levels after addition of pioglitazone for 12 weeks, (P=0.8545).

Lewis et al. [18]	2008	overweight (78 males and 128 females, Christchurch, New Zealand	A prospective cohort study, n = 206	-ELISA -Insulin resistance evaluated by HOMA-IR	Bivariate Spearman's correlation	RBP4 does not correlate with fasting glucose, insulin, or insulin resistance.
Erickstrup et al. [19]	2009	individuals with normal, impaired glucose tolerance and T2DM, Denmark	cross-sectional study, n = 233	-ELISA -Impaired glucose tolerance and T2DM evaluated by an oral glucose tolerance test	3- factor ANOVA	-When compared to people with normal glucose tolerance, the plasma concentration of RBP4 was lower in T2DM patients ($p<0.05$). -T2DM patients were compared to normal glucose tolerance participants, the ratio of RBP4 to retinol was found to be higher.
Ulgen F et al. [20]	2010	Non-diabetic obese subjects, (36 women, 34 men, body mass index >30 $kg/m^{2.}$), Germany	cross-sectional study, n=70	-ELISA -Glucose tolerance was assessed by oral glucose tolerance tests -Insulin resistance assessed by HOMA-IR	Multivariable linear regression adjusted for age and sex.	-Significantly higher RBP4 levels in obese men (53.0 +/- 20.8 microg/ml) than in obese women (39.7 +/- 12.3 microg/ml), ($p = 0.0013$). -No associations were observed between RBP4 and insulin sensitivity
Rhie YJ et al. [21]	2011	Children and adolescents (61 boys and 42 girls), between the age group of 6-18 years, all subjects grouped into 3 categories: groups (30 lean, 39 overweight, & 34 obese) according to the degree of obesity,	Cross-sectional study, n = 103	ELISA for RBP4	-For three groups, ANOVA test -between two groups, independent t-test. -Pearson's correlation	-Serum RBP4 levels of obese (60.44±16.13mg/L) and overweight groups(55.10±14.94mg/L) were higher than those of lean group (41.14 ± 21.30 mg/L). -There was no significant difference in serum

		korea			analysis	RBP4 (male Vs female as 54.94 ± 20.18 vs 49.67 ± 16.66 mg/L, P = 0.166). -RBP4 correlated significantly with BMI (r=0.567, p< 0.001).
Comucci EB et al. [2]	2014	females were evaluated into three major groups: Group 1(G1) (n = 45, Lean-control), Group 2(G2) (n = 53, obese), both having normal glucose tolerance, and Group 3(G3) (n = 41, obese with T2DM), Brazil	cross-sectional study, n= 139	-ELISA -Glucose tolerance was assessed by oral glucose tolerance tests -Insulin resistance assessed by HOMA-IR	-The comparison among three study groups (G1, G2, and G3) was performed by applying Kruskal Wallis test - correlation by Spearman correlation test	-Higher RBP4 levels (104.8 ± 76.8 ng/mL) were observed in G1 when compared to G2 (87.9 ± 38 ng/mL) and G3 (72.2 ± 15.6 ng/mL) levels. -a positive correlation of RBP4 with BMI (r = 0.253) in G1 -Glycated hemoglobin was positively correlated with RBP4 levels in all studied groups i.e., G1(r = 0.378), G2(r = 0.489) and G3(r = 0.330) -RBP4 levels were positively correlated with HOMA-IR in G3(r = 0.481)
Park et al. [22]	2014	Estimated RBP4 from 689 subject's serum and urine (21-80 years age group), with diverse glucose tolerance status as NGT (*n*=75), prediabetics, IGT (*n*=143), or Type 2 diabetics (*n*=471), Korea	Cross-sectional study, n= 689	ELISA Glucose tolerance was assessed by oral glucose tolerance tests	-ANOVA Test -Univariate regression analyses, and Pearson's correlation	- RBP4 levels(µg/ml) in urine were increased in prediabetics and Type 2 diabetics than in subjects with NGT i.e., T2DM (467.21±701.86) > prediabetes (391.08±723.33) >NGT (100.91±64.65),

						P<0.001 - Serum RBP4(µg/ml) & urinary RBP4 (µg/gCr) levels was found to be positively correlated with age (r= 0.139 & r=0.272), (p<0.001) & fasting plasma glucose (r=0.286 & r=0.241, p<0.001) respectively
Khalil H et al. [23]	2015	60 patients with type 2 diabetes mellitus, (28 men & 32 women), and 20 Healthy adults matched for age and BMI were chosen as controls, Cairo, Egypt	case-control study, n= 60	ELISA	-Student's t-test for comparison -Pearson's correlation for correlation	- T2DM patients had considerably higher blood RBP4 levels (53.227.57ng/ml) than non-diabetics (40.35 ± 5.02ng/ml). - serum RBP4 in males (57.18±7.58) was significantly higher than in females (49.76±5.69ng/ml) in diabetic patients Serum RBP4 levels in T2DM patients were positively correlated with age (r=0.281, p=0.030), fasting blood sugar (r=0.346, p=0.002), 2h post prandial blood sugar (r=0.389, p=0.0001), and HbA1c (r=0.464, p=0.0001), but negatively correlated with female gender (r=-0.435, p= 0.0001).
Grosjean F et al. [24]	2017	evaluated RBP4 serum levels in Hemodialysis subjects (*n* = 16)	Cross-sectional study,	ELISA	-ANOVA. -Pearson's	-serum RBP4 levels were raised in Hemodialysis subjects than that

		and matched healthy controls (n = 16), Italy	n= 32		correlation	in Controls (P < 0.005). -RBP4 levels correlated positively with fasting serum glucose (P < 0.05).
Jia-Ying li et al. [25]	2018	287 patients, aged 55–71 years, diagnosed with type 2 diabetes mellitus (diabetic retinopathy 83 & without DR 204), Guangzhou, China	Cross-sectional study, n= 287	ELISA	-Chi-Square or Mann–Whitney U-test logistic -linear regression models. -Spearman's rank correlation coefficient	- Patients with DR had substantially higher plasma RBP4 levels (39.7 (IQR: 29.8–47.7) g/ml than those without DR (25.0 (IQR: 15.5–32.6) g/ml; P<0.001. -plasma RBP4 levels were positively correlated with HbA1c (r = 0.347, P<0.001), BMI (r = 0.163, P=0.001) and duration of illness (r = 0.179, P<0.001). -plasma RBP4 levels were correlated with the incidence of diabetic retinopathy
Fan J et al. [26]	2019	-1,011 Chinese participants with prediabetes average age 55.6 ± 7.2 years). -Further investigated with 153 subjects with prediabetes developed type 2 diabetes, China	population-based prospective study, n=1,011	ELISA	Multivariate Cox regression model analysis to analyze the association of serum RBP4 levels and risk of incident T2DM	- Multivariate Cox regression model study revealed that serum RBP4 levels of 31g/ml and >55g/ml were linked to a greater risk of type 2 diabetes incidence. - U-shaped pattern, even low RBP4 concentrations are linked to an increased risk of diabetes in people with prediabetes.
Yeli Wang	201	571 incident type	Case-	Sandwich	The odds ratio	Men had

et al. [27]	9	2 diabetes cases and 571 controls, Singapore	control study, n = 571	ELISA	(OR) and 95 % confidence interval (CI) between RBP4 and the risk of type 2 diabetes mellitus were calculated using logistic regression models.	considerably higher plasma RBP4 levels than women, with median values of 30 (interquartile range [IQR]: 24–35) g/mL for men and 25 (IRQ: 21–31) g/mL for women.
Tan MI et al. [28]	2020	participants (>30 years old) were categorized into three categories (5 normal, 2 prediabetics, and 3 type 2 diabetic), Indonesia	Cross-sectional study, n = 10	Exosome Isolation and ELISA kit (Abcam)	-unpaired T-test - comparison of exosomal RBP4 between normal, prediabetic and diabetic followed two-tail ANOVA	-The concentration of free plasma RBP4 of diabetes participants (42.189mg/ml) was significantly increased than the healthy participant (35.125mg/ml), ($P<0.05$). -The concentration of exosomal RBP4 in blood plasma in healthy participants was higher (279.2±114.74ng/ml) than in prediabetic (226.5±53.03ng/ml) and type 2 diabetics (199.67±104.61ng/ml) -The concentration of exosomal RBP4 in urine (healthy 99.132ng/ml & diabetic 97.876ng/ml) was decreased than that of blood plasma. - RBP4 levels in urine were identical in diabetic and healthy subjects (7.000 and

						7.704ng/ml, respectively).

The above studies showed considerable findings, therefore, the present manuscript was designed to analyze free circulating levels of RBP4 in type 2 DM and predict its prime role in its pathogenesis.

Iron Homeostasis

Iron is an essential metal for hemoglobin synthesis of erythrocytes, oxidation-reduction reactions, and cellular proliferation. The total amount of body iron is ~3-4 g, two-thirds of which is composed of red blood cell iron and recycled iron by red blood cell destruction, and the remainder is stored in ferritin/hemosiderin, whereas only 1-2 mg of iron is absorbed in the intestinal tract and circulated in the blood. In circulation, iron is usually bound to transferrin, and most of the transferrin-bound iron is utilized for bone marrow erythropoiesis.[29]

Mechanisms Of Iron Toxicity

Being a transition element, iron has marked redox activity. The central importance of iron in the pathophysiology of the disease is derived from the ease with which iron is reversibly oxidized and reduced. This property, although essential for its metabolic functions, makes iron potentially hazardous because of its ability to participate in the generation of powerful oxidant species such as hydroxyl radicals. These free radicals are powerful pro-oxidants that may cause lysis of the lipid cellular membrane, damage the configurational harmony of proteins, and displace nucleic acids in genes. Such reactions can also contribute to tissue damage and increased oxidative stress thereby may potentially elevate the risk of type 2 diabetes mellitus.[30] (figure 3).

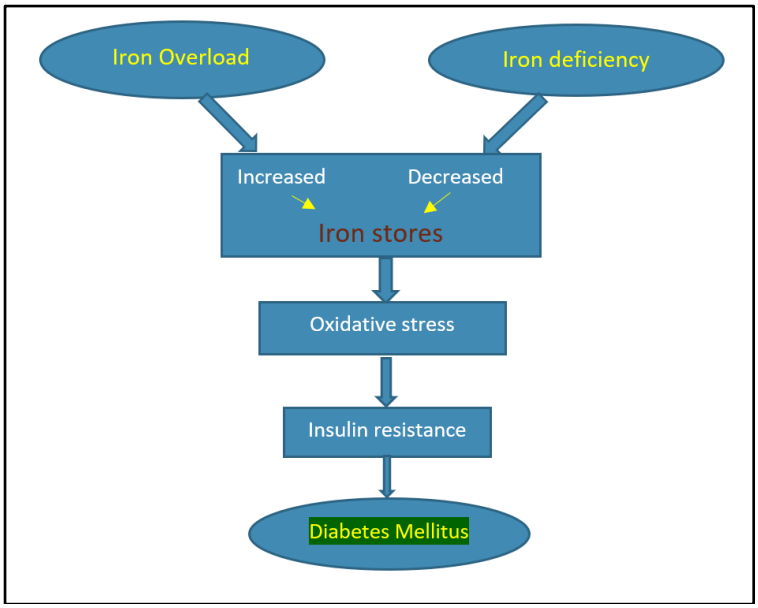

Figure 3: Iron interactions with insulin resistance and oxidative stress

An Overview Of Some Recent Studies Showing The Relationships Among Iron Parameters (Serum Iron, UIBC, TIBC, Serum Ferritin) In Type 2 Diabetes Mellitus Patients:

Name of the first Author	year	Study population/Region	Study Type & Sample Size	Method for quantification	Statistical methods	Main findings
Bansal P et al. [31]	2011	200 type II diabetes mellitus male patients, aged 35-65 years along with age & sex matched 200 healthy controls, Sharda Hospital, Greater Noida	Case-control study, n = 400	Automatic analyzer	Independent T-test	-The serum ferritin values were significantly increased in type II diabetic patients as compared to the control group 185 ± 3.5 ng/ml & 113 ± 4.6 ng/ml, respectively, ($p < 0.001$).
Zhao Z et al. [32]	2012	12 studies analyzed ferritin levels (4,366 T2DM patients and 41,091 controls) and 4 measured heme-iron intake (9,246 T2DM patients and 179,689 controls), Chengdu, China	systematic review and meta-analysis (prospective cohort studies or cross-sectional), n = 4366	MEDLINE database articles data	STATA v11.0 was used. All tests were two-tailed	Increased ferritin levels and heme-iron intake are both associated with a higher risk of Type 2 diabetes mellitus
Jung CH et al. [33]	2013	2,029 men without type 2 diabetes mellitus who underwent routine health	longitudinal observational study,	chemiluminescent two-site sandwich immunoassay for serum ferritin	-study population according to the ferritin quartile categories was compared using	-Elevated level of serum ferritin (185.8 ±109.4mg/dl) at baseline was associated with

		examination in 2007 (baseline) and 2011 (follow-up), korea	n = 2029		one-way ANOVA, -multivariate logistic regression analysis was used to calculate the relative risks (RRs) of the ferritin quartile categories for incident type 2 diabetes.	incident type 2 diabetes
Liu BW et al. [34]	2015	96 (male/female, 62/34) (aged 49.8 ± 11.9 years) subjects, divided into three groups according to the result of OGTT- Group NGT consisted of 32 normal glucose tolerant subjects (male/female 19/13, age 49.3 ± 10.9 years), Group IGR consisted of 33 impaired glucose tolerant and/or impaired fasting glucose subjects (male/female 22/11, age 50.1 ± 11.1 years), Group DM consisted of 31 newly diagnosed type 2 diabetes mellitus subjects (male/female 21/10, age 49.8 ± 11.2 years)	Cross-sectional study n= 96	-Glucose tolerance was assessed by oral glucose tolerance tests -Serum Ferritin was measured by electrochemilum inescence with model Elecsys 2010 immunity analyzer (Roche, German).	- ANOVA Test -Correlation analysis was made by Spearman test.	ferritin concentration in DM 2.2 ± 0.2 (μg/mL) higher than in IGR 2.0 ± 0.3 (μg/mL) when compared with NGT 1.8 ± 0.2 (μg/mL) subjects of no obesity -Serum Ferritin positively correlated with FPG (r = 0.451, P = 0.000)

		Qinhuangdao, China				
Misra G et al. [35]	2016	150 subjects which were divided into three groups (I, II, III) of 50 each. Healthy individuals (controls) constituted Group I, Group II consisted of T2DM patients with optimal glycaemic control (duration< 7 years, HbA1c<8%), T2DM patients with suboptimal glycaemic control(duration > 7 years, HbA1c>8%) formed group III. Chhatrapati Shahu Ji Maharaj University, Kanpur	case-control study, n= 150	-Blood glucose was measured by the glucose oxidase/peroxidase (GOD-POD) method, using a commercial kit from Span diagnostics (Surat, India) -Glycated hemoglobin was measured by the modified colorimetric method of Fluckiger. -Iron and TIBC were estimated by using a commercial kit (Ferrozine method) produced by Coral Clinical systems (Goa, India)	one-way ANOVA - Pearson correlation test	mean serum free iron concentration was 105.34 ± 3.5, 107.33 ± 3.45, and 125.58 ± 3.45 µg/dL in Group I, Group II, and Group III, respectively. Mean serum TIBC concentrations in Group I, Group II, and Group III were 311.39 ± 5.47, 309.63 ± 6.1, and 284.2 ± 3.18 µg/dL, respectively. Mean serum transferrin saturation (%) in Group I, Group II, and Group III was 34.17 ± 1.21, 35.02 ± 1.2, and 44.39 ± 1.07, respectively, (for all, p values < 0.0001) -a significant correlation was absent between serum iron and HbA1c (r = 0.05) and transferrin saturation (r = 0.0496) in Group III.
Wolide AD et al. [36]	2016	A total of 428 type 2 diabetes Mellitus patients and non-diabetic	comparative cross-sectio	-Fasting plasma glucose was determined by glucose hexokinase	Independent t-test for comparison.	serum concentration of ferritin 24.96±18.60(µg/L), and Fe+3

		study subjects aged 20-79 years, Ethiopia	nal, n= 428	method (ABX Pentra Glucose hexokinase 13CP kit), ferritin (ABX Pentra ferritin 5CP kit) was determined by radioimmunoassay method using automated chemistry analyzer ABX Pentra 400 (HORIBA Medical Diagnostics Instruments & Systems, Montpellier, France)		55.95±20.99 (µg/dL) in type 2 diabetic(µg/dL) patients was as compared to their respective controls' values of 34.99±18.53(µg/L) and 71.19±25.72(µg/dL) which was found to be significantly lower (p<0.0001)
Sowjanya Y et al. [37]	2017	120 subjects, age group 40-65 years, out of which 60 were taken as diabetics & 60 controls. Chinna Kakani, Guntur	A case-control study conducted, n = 120	-Glucose was analyzed in DADE Dimension automated system. -Ferritin was estimated by Automated Electro Chemiluminescence (ECLIA) method using a commercially available kit by ROCHE Cobas e411 - Iron & TIBC were estimated by RANDOX Imola. -HbA1c was tested by BIO-RAD D10 system which is based on the principle of High-	-Independent T-Test for comparison -Pearson correlation test for correlation between glycated hemoglobin and serum ferritin	-Serum Iron (µg/dl) in controls is 83.98± 22.89 while that of diabetic group is 67.38± 39.57 (<0.05), -TIBC (µg/dl) of a control group is 332.48 ± 65.89 as compared to diabetic which is 291.61± (65.86), (<0.05). -Also, Serum Ferritin (ng/ml) in controls is 51.07± 35.54 while the diabetic group is found to be 216.09±119.02, (P<0.05) -highly significant Positive

				Performance Liquid Chromatography (HPLC).		correlation is observed between Ferritin & HbA1C levels in Type 2 Diabetes with (r =0.6083) and (P=0.001).
Kumar S et al. [38]	2018	60 diabetic patients & 60 controls, were categorized into three groups. They were: Group 1 (Healthy controls, matched for age and gender, with HbA1C <6.5%), Group 2 (Individuals with diagnosed type 2 diabetes mellitus patients with HbA1C 8% / without complications) and Group 3 (Individuals with diagnosed Type 2 Diabetes mellitus patients with HbA1c > 8% / with complications). Government Medical College Jammu, J&K, India	Case-control study, n =120	-Blood glucose was estimated by Abott ARCHITECT c-Systems by hexokinase method -HbA1C was estimated on ARCHITECT c-Systems -Ferritin levels were measured by chemiluminescent microparticle immunoassay (CMIA)	Independent T-Test for comparison -Pearson correlation test for correlation	-The mean serum ferritin level was found to be higher in cases as 293.9±20.6 ng/ml for group 3 and 168.5±11.8 ng/ml for group 2 as compared to controls (Group 1) with mean values of 100.6 ± 7.0 ng/ml, (p<0.001) -Ferritin was positively correlated with HbA1c and was significant in group 2 and group 3 as well.
Dhakad GS et al. [30]	2019	100 subjects were taken in this study, out of which 50 were type 2 diabetes and 50	case-control study, n = 100	-The serum iron, TIBC, UIBC, plasma glucose, and HbA1c were measured by a	Z test was used for comparison	- The serum iron (134.66 ±42.06 in cases,111.84 ±21.45 in controls), and

		were normal healthy controls, Gwalior (Madhya Pradesh)		semi-auto analyzer. -The serum ferritin was estimated by the chemiluminescence method. -The % transferrin saturation was calculated by the formula serum iron (µg/dl) x 100/ TIBC (µg/dl).		serum ferritin (400.24± 64.28 in cases, 190.94 ±55.64 in controls) were statistically extremely significantly ($p<0.001$) increased - UIBC (165.5± 70.57in cases, 214.34 ±65.29 in controls) was highly statistically significantly ($p<0.001$) decreased while - TIBC (300.16±79.0 in cases, 326.18 ±46.92 in controls) and hemoglobin were statistically significantly($p<0.05$) decreased in type 2 diabetes mellitus subjects as compared to healthy control subjects. -% saturation was statistically extremely significantly ($p<0.001$) increased in (42.08±15.39 in cases & 35.7±10.91 in controls)
Son NE et al. [39]	2019	172 patients, 84 of whom had type 2 DM and	cross-sectional,	Roche COBAS Integra 400 automated	-Independent samples t-test	serum ferritin levels of type 2 DM diabetics

		88 without diabetes and constituted the control group, Western Turkey	controlled study, n= 172	analyzer	-Spearman's correlation coefficient	were significantly increased, 81.27±37.20 ng/ml as compared to their controls 63.69±17.00, (p<0.001). -A strong positive correlation was found between serum ferritin levels and HbA1c and fasting blood glucose (FBG) levels (p<0.01)
Moirangthem MM et al. [40]	2020	270 type 2 diabetes subjects (equal number of Male & Female - 135:135, mean age 46.40 ± 9 years) were studied and compared with 30 controls (mean age 47.27 ± 11 years). Imphal, Manipur	Cross-sectional study, n= 270	Serum ferritin was performed on the LIAISON® Analyzer by sandwich chemiluminescence immunoassay. 2. Glycated hemoglobin (HbA1c) done using kit supply by Medsource Ozone Biomedicals Pvt. Ltd by Ion Exchange Resin Method 108	-Independent t-test -Pearson correlation	-The mean serum ferritin level of the diabetic patients was significantly higher (172.7±11ng/dl), (p<0.013) than that of the controls(102.5±7ng/dl). -There was a positive correlation (r = 0.036) (p-0.0392) between HbA1c and serum ferritin among diabetic patients.
Sumathi K et al. [41]	2020	200 subjects consisting of 100 cases and 100 controls. The Cases group included 100 iron-deficiency anemia patients with diabetes	Case-control study, n = 200	HbA1c levels were measured by ion exchange chromatography, -ferritin by particle enhanced turbidometric immunoassay	-Students t-test	-HbA1c was elevated (7.3±0.9%) in iron-deficient diabetic individuals compared to normal level (5.4±0.6%) in controls,

		having controlled plasma glucose levels and Controls included 100 non-anemic diabetic individuals, Chennai		method, -fasting plasma glucose by GOD - POD method.		(p<0.01) -serum ferritin was lowered in cases group (6.5±3.5 ng/ml) as compared to controls (235±75.5 ng/ml).
Michea l S et al. [42]	202 1	A total number of 50 type 2 diabetic cases and 50 controls, 35 -60 yrs. were taken in this study, Chennai	Case-contro l study, n = 100	-ferritin by particle enhanced turbidometric immunoassay method,	-students t-test. -Pearson correlation	-serum ferritin levels mean were significantly higher in diabetes 120.44ng/ml than in the control group 62.27ng/ml. -positive correlation was found between serum ferritin and HbA1c in diabetic patients (0.34), between ferritin & FBS is 0.412, and ferritin & PPBS is 0.315.
Vasava SN et al. [43]	202 1	50 type 2 diabetes mellitus cases of iron deficiency anemic individuals with controlled plasma glucose levels and 50 non-anemic non-diabetic individuals, Gujarat	Case-contro l study, n = 100	-HbA1c was analyzed by HPLC method using Hb Vario HbA1C analyzer. -Plasma glucose was analyzed by glucose oxidase/peroxid ase method using a fully automated chemistry analyzer EM 200. - Serum ferritin was analyzed by Nephlometry	-student's t-test -Categorical data were analyzed by x2 test.	-HbA1C levels (6.90 ± 0.81%) were increased in iron-deficient individuals as compared to non-anemic (5.17 ± 0.54%). --HbA1C (7.08 ± 0.78%) was elevated in females than in males (6.67 ± 0.81%) in cases group. -HbA1C was elevated more in subjects having FBS between

				method on Mispa- i2 analyzer.		100-126 mg/dl (prediateic) (i.e.,7.31 ± 1.52%) compared to the those with normal FBS (<100 mg/dl) of HbA1c 6.83±1.02%. -HbA1c levels were significantly higher in subjects above 50 years of age compared to the subjects below 50 years of age.

Hence, in view of the above studies, iron status needs to be monitored at regular intervals in the diagnosis and management of type 2 Diabetes mellitus patients so that earlier appropriate measures can be taken to avoid iron deficiencies.

A Possible Outcome Of The Relationship Between RBP4 And Iron Status

RBP4 is found to be an indicator of vitamin A (retinol) intake. As suggested in some studies, vitamin A deficiency can impair iron metabolism and may further aggravate anemia. Iron supplementation, on the other hand, dramatically enhanced plasma retinol and RBP4 levels.

Based on this, Fernandez-Real et al. (2008) at Girona, Spain evaluated, in a cross-sectional study, insulin resistance, serum ferritin & serum RBP4 levels in 132 individuals (non-diabetic, middle-aged men) and in a replication-independent study. They also observed blood RBP4 levels in type 2 diabetic patients before and after iron deficiency. Finally, the effect of iron in RBP4 release in adipose tissue was examined in vitro. A competitive enzyme-linked immunosorbent test was used to study circulating RBP4 in supernatants (AdipoGen, Seoul, Korea).

They discovered a positive relationship between circulating RBP4 and log serum ferritin in both studies ($r = 0.35$ and $r = 0.61$, respectively; $P < 0.0001$). Serum RBP4 concentrations were observed to be higher in males than in women ($P = 0.001$), which coincided with higher ferritin levels ($P < 0.0001$).

Log serum ferritin contributed significantly to RBP4 variance in multiple regression studies to predict serum RBP4. In type 2 diabetes individuals, plasma RBP4 levels were found to be reduced following iron depletion (percent mean difference -13.7 [95 percent CI -25.4 to -2.04]; $P = 0.024$). Based on their findings, they summarized the connection between circulating RBP4 and iron stores, both cross-sectional and after iron depletion, and in vitro, and concluded that the

association between RBP4 and insulin resistance might be explained by iron overload to some extent.[8]

Sun L et al. (2014) in a prospective study evaluated a total of 2091 Chinese adults aged 50–70 years and were followed up for 6 years. They observed that Plasma RBP4 was positively correlated with ferritin in both men (r= 0.08) as well as women (r=0.14), p<0.001. Plasma RBP4 concentration was significantly associated with dietary heme iron intake, plasma ferritin concentration, and other established risk factors, and RBP4 is independently associated with the future development of type 2 diabetes mellitus.[9]

Conclusion

The relationship between circulating RBP4, RBP4 release in adipose tissue explants after iron modulation, and reduced serum RBP4 after iron depletion, based on studies suggested that in the RBP4–insulin resistance association, iron may have a crucial role. However, the mechanism behind the link between retinol-binding protein-4 (RBP4) and insulin resistance, is still being investigated. Interaction between iron and vitamin A status, of which RBP4 is a surrogate, has recently been recognized and whether iron overload could contribute to the RBP4–diabetes association has not been evaluated in-depth previously. Therefore, it is necessary to further investigate whether the association of serum RBP4 with insulin resistance in type 2 diabetes patients could be due to increased iron stores or not and also highlights the possible related mechanism.

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