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FERRIC STATUS AS A BIOCHEMICAL INDICATOR ASSOCIATED WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Background: Epidemiological studies have shown that Diabetes Mellitus is an cronic inflammatory condition. The increased iron stores are associated with increased free radical formation, which contributes to the glucose intolerance and consequently diabetes type 2. The objective of this study was to evaluate the parameters of the ferric status in patients with type 2 diabetes mellitus. **Methods:** The study group consisted of 100 blood samples (50 males and 50 females) of healthy individuals and 69 blood samples (28 males and 41 females) from individuals with type 2 diabetes mellitus. All samples were screened for the possibility of presenting any condition that could interfere in the determination of ferric status parameters. **Results:** The results show that the mean serum ferritin concentration in the diabetic group is higher than the control group mean when treated without distinction of sex (228.06 mg/mL × 126.26 mg/mL) and with in relation to the sex (200.96 mg/mL × 122.79 mg/mL for *females* and 267.84 mg/mL × 129.73 mg/mL for *males*) demonstrating a statistically significant difference between the two study groups ($p < 0.001$). The statistical test also showed that SFe (Serum Ferric), TIBC (Total Iron Binding Capacity) and TSI (Transferrin Saturation Index) presented significant difference between the control and diabetic groups [without gender distinction ($p < 0.001$)] and with respect to this parameter, [significant difference in relation to the female sex ($p < 0.001$)]. **Conclusions:** The results showed that elevated iron stores are more common in diabetic patients suggesting that there is a positive correlation between the excess of organic iron and predisposition to develop type 2 diabetes and/or possibly its complications. Individuals with hereditary hemochromatosis (excess iron in the body) naturally develop diabetes, from which arose the need to investigate iron metabolism as a conditioner for metabolic syndrome or diabetes. In this study, was possible to observe a positive correlation between the iron stock measured by serum ferritin concentration and type 2 diabetes mellitus. The concentration of ferritin is still associated with components considered as a cardiovascular risk factor such as elevated glucose concentration (pre-diabetes and diabetes), LDL cholesterol, triglycerides and HDL cholesterol.

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INTRODUCTION

The Iron is one of the most abundant metal in the human body and essential micronutrient for most living organisms, due to it their oxy-reductive capacity in a variety of enzymes and proteins heme and non-heme (Rines, 2013). Clara Podmore *et al.*, 2016 in "Association of multiple biomarkers of iron metabolism and type 2 diabetes: the EPIC-interact study.

(Report)" and Fernandes-Real *et al*., 2015 in "Mechanisms Linking Glucose Homeostasis and Iron Metabolism Toward the Onset and Progression of Type 2 Diabetes". Considering the results these studies the objective of this work is to relate iron to the development of type 2 diabetes because are studies that make the clinical relationship between iron metabolism and the development of type 2 diabetes (Clara Podmore, 2016 and José Manuel Fernández-Real, 2015). In biochemical terms the pancreatic damage was observed because excess of iron in beta cells is a strong predictor of toxicity (Noetzli, 2012).

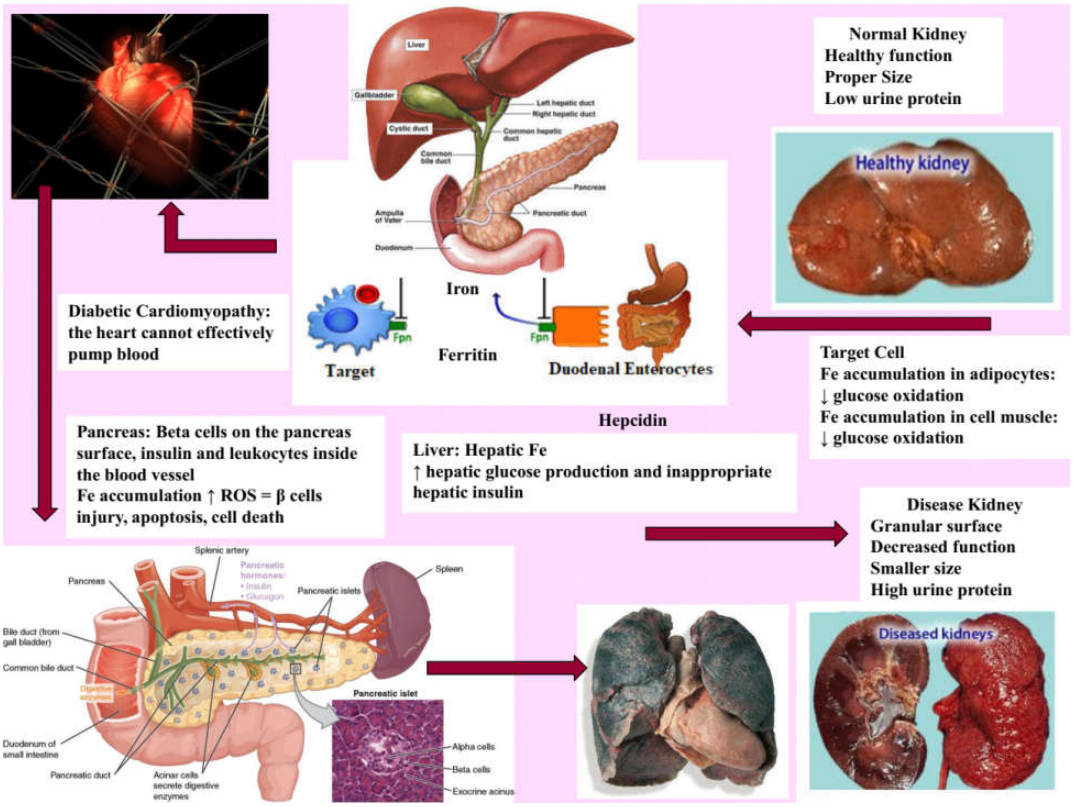


Figure 1. Schematic showing the systemic effects of iron accumulation, diabetes and its complications

It is also observed that pre-diabetic patients despite have a low risk of mortality, this risk is compounded when they have associated with high levels of serum iron (Mainous, 2014), demonstrating the need to assess iron stores in patients with abnormal glucose (Orban, 2014). Intestinal free ferric iron (Fe^{3+}) is reduced to (Fe^{2+}) by the ferrireductase duodenal cytochrome b (DCTB) and enters the cell through the divalent metal-ion transporter 1 (DMT1) and possibly other carriers. Dietary heme is directly absorbed and iron is released by heme oxygenase (HMOX). Iron exits the enterocyte through the iron export channel ferroportin (FPN). After oxidization by hephaestin (HEPH) iron binds to transferrin (Tf) and in the bloodstream which binds to transferrin receptors (TfR) 1 and 2 on the surface of target cells. Ferritin secreted into the blood serves as a marker for tissue iron stores. Hepcidin induces internalization and degradation of (FPN), thus completing a negative feedback regulatory loop. Although iron overload is associated with diabetes risk, iron deficiency is associated with another important risk factor for diabetes, the obesity (Simcox, 2013 and Arija, 2014). See Figure 1. This oxidative stress contributes to the development of insulin resistance and beta cell (pancreas) dysfunction, also to potentiating the risk of cardiovascular diseases, liver diseases, kidney diseases, lung diseases. The metabolic imbalance affects the organism in a systemic way, because through the circulation it reaches all the organs and viscera.

Font: Simcox, J. A., & McClain, D. A. (2013). Iron and Diabetes Risk. *Cell Metabolism*, *17*(3), 329–341. http://doi.org/10.1016/j.cmet.2013.02.007; Barnes PJ (2010) Chronic Obstructive Pulmonary Disease: Effects beyond the Lungs. PLoS Med 7(3): e1000220; https://doi.org/10.1371/ journal.pmed.1000220; https://hubtiva.com/six-6-tips-for-reducing-the-risks-of-kidney-disease/; https://www. shutterstock.com/pt/image-illustration/beta-cells-on-pancreas-surface-insulin-205144576?src=RyXUDsBhGbKR1tXQH0tOVg-1-12; https://www.medicinenet.com/liver_disease/article.htm; https://www.medscape.org/viewarticle/876836_2; http://www.bloodjournal.org/content/123/2/168?sso-checked=true; https://courses.lumenlearning.com/suny-ap2/chapter/the-endocrine-pancreas/; https://www.drterrywillard.com/the-liver-our-great-alchemist-part-1/

The accumulation of this metal in the tissues can lead to the development of diseases and damages in several organic systems, such as hepatic, cardiac, endocrine and musculoskeletal (Carrondó, 2003; Testa, 2002 and Andrews, 2005). Evidence suggests that hyperglycemia is associated with the acceleration of lipid peroxidation by inducing the body to a chronic inflammatory state and non-enzymatic glycation of LDL cholesterol. In this context, several epidemiological studies have demonstrated the association between increased iron stores and the development of type 2 diabetes mellitus and its complications (Powell, 1998; Witte, 1996; Redmon, 1994; Ceriello, 2004; Tuomainen, 1997; Ford, 1999 and Fernandez-Real, 2002). Some studies report that the excessive deposition of iron in the pancreas can lead to the development of diabetes, because it damages the beta cells and causes a change in the response pattern of these cells in the production and secretion of insulin, leading to insulin resistance. For example, 80% of patients with hereditary hemochromatosis develop type 2 diabetes mellitus (Fernandez-Real, 2002; Thomas, 2004 and Ikeda, 2006). See figure 1. Iron

catalyzes the oxidation of lipids and proteins and the formation of reactive oxygen species (ROS), such as the hydroxyl radical (OH^-) and superoxide anion (O_2^-), which damage cell macromolecules, which can promote cell death and tissue injury (Tuomainen, 1997; Fernandez-Real, 2002; Emerit, 2001; Papanikolaou, 2005). Iron is closely linked to oxidative stress, via Fenton's reaction, which can induce insulin resistance, by decreasing its internalization and increasing ferritin synthesis (Fernandez-Real, 2002; You, 2005). According to MacDonald, *et al*. 17 (MacDonald, 1994), the expression of H-ferritin mRNA is 4 and/or 8 fold higher in beta cells of diabetic rats compared to non-diabetic rats and that beta cells are particularly sensitive to ROS. Considering the data presented and reports in the literature (Arija, 2014; Tuomainen, 1997; Hansen, 2014; Friedwald, 1972; Paiva, 2000; Tuomainem, 1998 and Ramakrishnan, 2002), the objective of the present study was to verify the parameters of the ferric status in patients with type 2 diabetes mellitus and analyze possible correlation between them.

MATERIALS AND METHODS

The study material consisted of blood samples from two groups of volunteers: diabetes mellitus type 2 patients (diabetic group) and healthy people (control group). The data on smoking, physical activity, giving and receiving blood; drug use, alcohol consumption and medical history were obtained at the time of the interview, in which they received appropriate guidance on the objectives of the study and signed the Informed Consent Term (ICT) for participation. The procedures performed in this study were approved by the Ethics Committee of the Hospital Universitário Presidente Dutra da Universidade Federal do Maranhão (HUUFMA), under number 56943. Blood samples were obtained by venipuncture, after fasting for approximately 12 hours and distributed in tubes with and without anticoagulant (EDTA-Ethylenediamine Tetraacetic Acid). The tube containing EDTA was for realization of complete blood count in micro device 60 (ABX), and without anticoagulant was subjected to centrifugation to separate the serum and subsequent implementation of biochemical tests (lipid profile and glucose, liver enzymes, ferric status parameters and C reactive protein - CRP). The determination of Blood Glucose, Total Cholesterol, HDL Cholesterol and Triglycerides was carried by enzymatic methods endpoint; The values of LDL Cholesterol was obtained by the Friedewald equation and coworkers [27], when the concentration of triglycerides was less than 400 mg/dL; the concentration of liver enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma glutamyl transferase) were obtained by kinetic enzymatic methods. The Serum Iron levels and Total Iron Binding Capacity were obtained by colorimetric endpoint methods; Transferrin Saturation Index was obtained by calculation (SI/TIBC x 100); Serum Ferritin was determined by turbidimetric method and the CRP for latex particle agglutination. In order to minimize possible interferences in the analysis of the laboratory results of the study groups, especially with regard to the concentration of ferritin, which may arise in situations such as infection, liver diseases and alcohol intake (Fernandez-Real, 2002), the following criteria were applied: (ALT> 74 U/L, AST> 68 U/L, ALP and γGT> 1.5 times the normal value), which were positive for CRP (concentration> 6 mg/L), high concentrations of hepatic enzymes are indicators of inflammatory processes and liver diseases, respectively (Thomas, 2004 and Ikeda, 2006). ingestion of alcohol 48 hours prior to collection, presence of iron deficiency anemia or iron depletion/deficiency, use of vitamin supplements and/or medicinal products containing iron, donation or receipt of blood in the last three months; increased number of circulating leukocytes (> 12,000/mm^3 of blood); presence of anemia (hemoglobin concentration <12 g/dL for women and <13 g/dL for men); presence of iron deficiency, according to the criteria established by Cook *et al*. (Cook, 1982) and hemolyzed samples. Statistical analysis for comparison between groups was performed using the Wilcoxon-Mann-Whitney Test. The statistically significant value was considered according to a level of significance of 95% ($p < 0.05$).

RESULTS

After the processing of the samples and analysis of the results, the exclusion criteria were applied, which resulted in a discard of 67 (28.4%) of the samples from the study group (27 males and 40 females). Thus, the study group consisted of 169 volunteers, with mean age of 62.34 years for females and 58.19 years for males. A total of 69 (40.83%) samples belonged to the diabetic group (41 females and 28 males) and 100 (59.17%) samples belonging to the control group (50 females and 50 males). The distribution of the laboratory parameters analyzed in this study are shown in Table 1.

Table 1. Averages of laboratory results and statistical analysis according to Wilcoxon-Mann-Whitney

Parameters	Group of Diabetics (n = 69) X and SD (mg/dL)	Group Control (n = 100) X and SD (mg/dL)	*(p)*
Glucose	132,21 (± 58,10)	86,57 (± 11,99)	< 0,001
Total Cholesterol	200,86 (± 52,81)	197,64 (± 30,03)	NS
Cholesterol HDL	40,19 (± 8,75)	47,74 (± 7,13)	< 0,001
Cholesterol LDL	132,78 (± 51,78)	118,99 (± 32,54)	0,017
Triglycerides	141,94 (± 56,24)	148,57 (± 86,27)	NS
Serum Iron	109,55 (± 79,26)	128,85 (± 28,32)	< 0,001
CTLF	330,32 (± 100,84)	299,45 (± 61,31)	NS
Serum Ferritin	228,06 (± 154,01)	126,26 (± 73,26)	< 0,001
TSI	38,05 (± 29,21)	46,22 (± 21,08)	NS

NS: Not significant; TSI: Transferrin Saturation Index; TIBC: Total Iron Binding Capacity; HDL: High Density Lipoproteins; LDL: Low Density Lipoproteins. The Wilcoxon Mann-Whitney Test is one of the most powerful of the nonparametric tests for comparing two populations

Analysis of ferric status parameters (Figure 2) showed that there is a positive association ($p < 0.001$) between serum iron and type 2 diabetes mellitus, with respect to the treated group regardless of sex and with relation female sex. Regarding the total iron binding capacity, values were statistically significant for females ($p < 0.001$) and the same for transferrin saturation index ($p < 0.001$). For the serum ferritin, which represents the iron stores, a positive association was observed between the elevation of its concentration and the group with diabetes mellitus, in both sexes and when the groups were treated without gender distinction ($p < 0.0001$). Regarding the parameters of the ferric status, it was observed in the diabetic group that: 15.90% (05 female and 06 male) presented elevation of serum iron; 21.71% (13 females and 02 males) had elevated total iron binding capacity and 15.90% (05 females and 06 males) showed elevated transferrin saturation index. Regarding serum ferritin, was observed that 56.52% of the subjects in the diabetic group (27 females and 12 males) presented high concentrations. In the control group, serum iron was found to be elevated in 17% of the volunteers (06 female and 11 male) and 32% (16 female and 16 male) had elevated

transferrin saturation index. Regarding serum ferritin, was observed that 24% of the individuals (20 females and 04 males) presented concentrations above the reference values. (Table 2 and Figure 2).

Table 2. Percentage of laboratory results and statistical analysis according to Wilcoxon-Mann-Whitney

Parameters Figure 2	Group of Diabetics (n = 69)	Group Control (n = 100)	*(p)*
Serum Ferric	15,90%	17%	<0.001
TIBC	21,71%	0%	<0.001
TSI	15,90%	32%	<0.001
Serum Ferritin	56,52%	24%	<0.0001

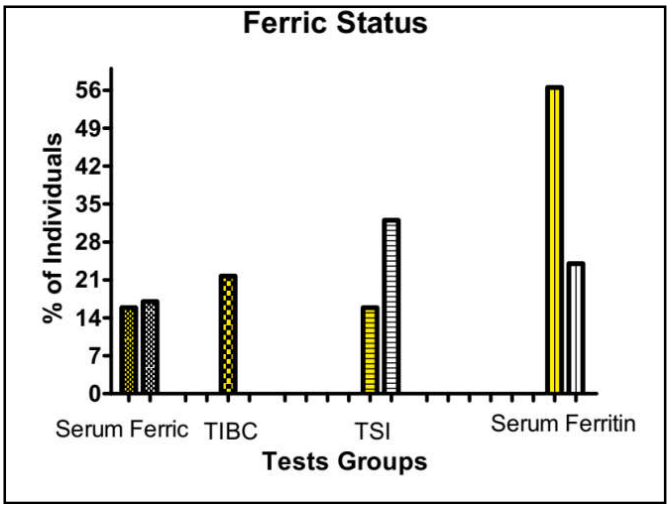


Figure 2. Laboratory Screening (Ferric Status)

The graph above shows that serum ferritin was statistically high in the group of diabetic patients compared to the control group of non-diabetic patients. TIBC: Total Iron Binding Capacity; TSI: Transferrin Saturation Index; Yellow = Diabetic Group; White = Control Group. In the lipid profile of the diabetic group, 10 (14.49%) had total cholesterol above the recommended values, being that all belonged to the female sex. HDL cholesterol was below the reference values in 28 (40.57%) patients, being that 15 were female and 13 were male. LDL cholesterol was elevated in 13 (18.84%) volunteers, being 08 females and 05 males. The value of triglycerides was high in 10 (14.49%) subjects of the research, being 07 females and 03 males. In the control group, in relation to the lipid profile, it was verified that 07 (07%) volunteers had high total cholesterol, of which 02 were female and 05 were male; HDL cholesterol was decreased in 11 (11%) volunteers, of whom 07 were female and 04 were male. Regarding LDL cholesterol, it was observed that 09 (09%) volunteers presented values above those recommended, of which 02 were female and 07 were male. The concentration of triglycerides was high in 18 (18%) of the subjects, of which 4 were female and 14 were male. (Table 3 and Figure 3).

Table 3. Percentage of laboratory results for Lipid Profile

Parameters Figure 3	Group of Diabetics (n = 69)	Group Control (n = 100)
Total Cholesterol	14,49%	7%
Triglycerides	14,49%	18%
HDL	40,57%	11%
LDL	18,84%	9%

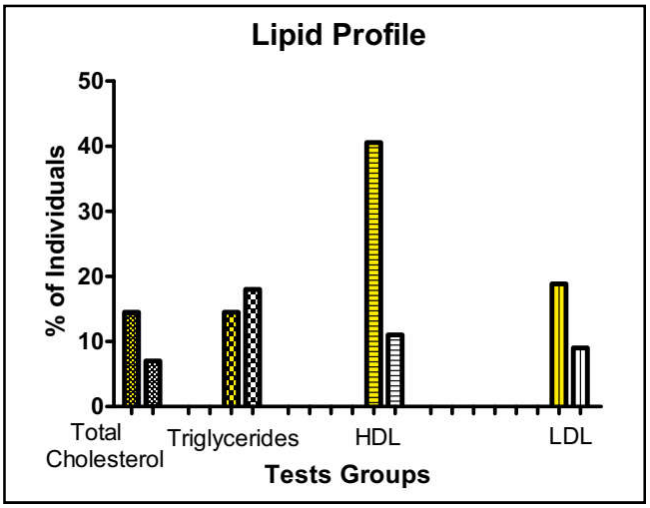


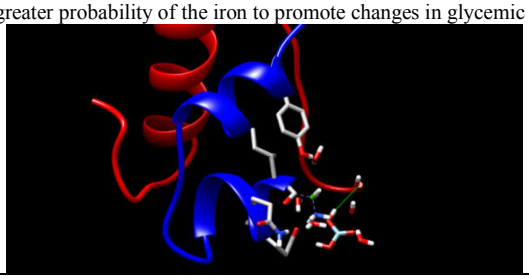
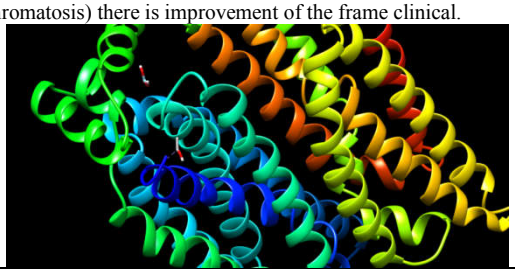
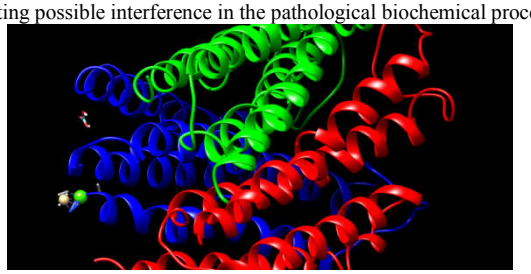
Figure 3. Laboratory Screening (Lipid Profile)

The chart above shows that the lipidogram in the group of diabetic patients was reasonably high. This increases oxidative stress because iron catalyses lipids by increasing insulin resistance as explained in the introduction. HDL: High Density Lipoproteins; LDL: Low Density Lipoproteins; Yellow = Diabetic Group; White = Control Group. The diabetic patients belonging to the study group were analyzed for glycemic changes, and it was possible to verify that 49.27% of the patients had a high glucose level (19 females and 15 males) and it was still possible to observe that ferritin is positively associated with the elevation of glucose concentration in this specific group.

DISCUSSION

In this study, it was possible to observe a positive correlation between the iron stock measured by serum ferritin concentration and type 2 diabetes mellitus. The concentration of ferritin is still associated with components considered as a cardiovascular risk factor such as elevated glucose concentration (pre-diabetes and diabetes), LDL cholesterol, triglycerides and HDL cholesterol (Viebig, 2006). Dyslipidemia is an important risk factor for the development of coronary artery disease or even appears to be a prerequisite for coronary artery disease and, in some cases, would precede other cardiovascular risk factors (Feio, 2003). Cardiovascular diseases are the most frequent cause of morbidity and mortality in Brazil (300.000 deaths/year), second data of Pan American Health Organization (OMS). The disorder of lipid metabolism is 2 to 3 times more frequent in individuals with type 2 DM than in non-diabetics. The prevalence of dyslipidemia in diabetics is around of 35% and abnormalities in lipoprotein metabolism are characteristic of this patients when compared with nondiabetic individuals; higher triglyceride concentrations in both the cases, fasting and basal, and the postprandial increase in triglyceride-rich lipoproteins (VLDL - very low density lipoproteins) and these findings are still accompanied by lower concentrations of HDL cholesterol. Although the concentration of LDL cholesterol may not be increased in type 2 diabetic patients, its metabolism is abnormal, with a tendency towards greater oxidation and glycosylation (MacDonald, 1994). The mechanisms that lead to the association between diabetes in individuals with high iron stores are not well understood.

Table 4. Molecular Docking between iron ligands and targets associated with the development of diabetes

Ligands	**PubChem Links**
1. FerrousCarbonate 2. Ferrous Fumarate 3. Ferrous Succinate	https://pubchem.ncbi.nlm.nih.gov/compound/ferrous_carbonate https://pubchem.ncbi.nlm.nih.gov/compound/5478817 https://pubchem.ncbi.nlm.nih.gov/compound/Ferrous_succinate
Ligation of Energy Full Fitness (kcal/mol)	**Targets Link PDB**
	Human Insulin http://www.rcsb.org/pdb/explore/explore.do?structureId=3I40
1. -346.09 (kcal/mol)* $FeCO_3$ 3. -328.34 (kcal/mol) $C_4H_4FeO_4$ 2. -325.33 (kcal/mol) $C_4H_2FeO_4$	**Ferrous Carbonate, SwissDocking Cluster #0, Element 0, FullFitness: -346.09 kcal/mol, ΔG=-5.40 kcal/mol.** **Discussion:** * The higher value of FullFitness negative represents the greater interaction target-ligand, therefore greater probability of the iron to promote changes in glycemic metabolism. 
	Human glucose transporter GLUT1 http://www.rcsb.org/pdb/explore/explore.do?structureId=4PYP
1. -1274.68 (kcal/mol)* $FeCO_3$ 3. -1258.25 (kcal/mol) $C_4H_4FeO_4$ 2. -1255.44 (kcal/mol) $C_4H_2FeO_4$	**Ferrous Carbonate, SwissDocking Cluster #0, Element 0, FullFitness: -1274.68 kcal/mol, ΔG=-6.0 kcal/mol.** **Discussion:** * The higher value of FullFitness negative represents the greater interaction target-ligand, therefore greater probability of the iron to promote changes in glycemic metabolism. The GLUT1 protein occupied by the GLUT1-Iron interaction not will may to perform its normal functions. GLUT 1 is a glucose transport protein present in blood cells, blood brain barrier and kidneys, we can observe that high interaction between iron carbonate and GLUT1 can be a plausible explanation for when occurs phlebotomy (venous bleeding - iron withdrawal: hereditary hemochromatosis) there is improvement of the frame clinical. 
	Human microsomal prostaglandin E synthase 1 http://www.rcsb.org/pdb/explore/explore.do?structureId=3DWW
1. -2064.42* (kcal/mol) $FeCO_3$ 3. -2046.08 (kcal/mol) $C_4H_4FeO_4$ 2. -2044.47 (kcal/mol) $C_4H_2FeO_4$	**Ferrous Carbonate, SwissDocking Cluster #0, Element 0, FullFitness: -2064.42 kcal/mol, ΔG=-6.60 kcal/mol.** **Discussion:** Ferrous Carbonate is the binder that has the highest negative binding energy with the target Human microsomal prostaglandin E synthase 1. Every stable system tends to the minimum of energy to exist in nature, it will have higher negative bond energy to the patterns of the classical physics, soon the interaction (Human microsomal prostaglandin E synthase 1-Ferrous Carbonate) shows a high degree of affinity, high probability of the interaction occurring in practice, indicating possible interference in the pathological biochemical process of diabetes. 

Evidence indicates the oxidative mechanism with the generation of reactive oxygen species (ROS), because they are directly associated with changes in the action of insulin and lack of control of blood glucose concentration (Reaven, 1988), other studies suggest that iron deposition in the pancreas (beta cells) contributes to insulin resistance because it affects the synthesis and secretion of this hormone (Jiang, 2004), others still report that the deposition of this metal in the muscles decreases the uptake of glucose in these cells due to muscle damage (Andrews, 2005; Witte, 1996; Hansen, 2010).

It is known thatiron stores contribute to metabolic alterations, because it is a transition metal that participates in oxidation-reduction reactions, which can lead to tissue damage and exacerbation of oxidation of lipoproteins by the generation of reactive oxygen species (Testa, 2002 and Andrews, 2005). The table following shows an *in silico* experiment between iron ligands and targets related to glucose metabolism (human insulin, GLUT1 receptor and prostaglandin E1), where high affinity was observed (maximum negative binding energy - see table 5). These results suggest the same line of reasoning

Supplement 1|- List of clinical trials emphasizing the aspects of metabolism of the Figure 1

Title	Results	DOI
Circulating ferritin concentrations and risk of type 2 diabetesin Japanese individuals.(Report)	The results suggest that elevated iron storage is associated with increased risk of type 2 diabetes in normal weight individuals and that this association is partly mediated through liver dysfunction resulting in insulin resistance.	doi: 10.1111/jdi.12617
Increased Small Intestine Expression of non-Heme IronTransporters in Morbidly Obese Patients With Newly Diagnosed Type 2 Diabetes	Increased intestinal iron absorption is a potential mechanism which could explain the increased body iron stores frequently observed in patients with Type 2 Diabetes.	doi: 10.1002/mnfr.201700301
Serum copper, zinc, and iron levels, and markers of carbohydrate metabolism in postmenopausal women with prediabetes and type 2 diabetes mellitus	A significant elevation of total metal concentration in diabetic subjects without a concomitant elevation of transport proteins may be indicative of increased levels of Fe and Cu.	Doi: 10.1016/j.jtemb.2016.11.005
Iron: a Strong Element in the Pathogenesis of Chronic Hyperglycaemia After Acute Pancreatitis	These findings suggest that iron metabolism is significantly altered in individuals with chronic hyperglycaemia after acute pancreatitis	doi: 10.1007/s12011-017-1131-y
Serum ferritin concentration in early pregnancy and risk of subsequent development of gestational diabetes: A prospective study	High serum ferritin can be considered as a significant risk factor for the development of gestational diabetes.	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5447832/
Plasma ferritin, C-reactive protein, and risk of incident type2 diabetes in Singapore Chinese men and women	The elevation of blood ferritin levels, in the presence of raised high sensitivity C reactive protein, was significantly associated with increased risk of type 2 diabetes.	doi: 10.1016/j.diabres.2017.04.012
Simple sugar supplementation abrogates exercise-induced increase in hepcidin in young men	The data suggest that protective effects of exercise on excess iron accumulation which is mediated by hepcidin can be abrogated by high sugar consumption.	doi: 10.1186/s12970-017-0169-8
High serum ferritin levels are associated with insulin resistance but not with impaired glucose tolerance in a healthy people population	Serum ferritin could be considered as an early marker of insulin resistance prior to the onset of glycemic disorders.	doi: 10.1016/j.dsx.2017.07.026
Trace elements in early phase type 2 diabetes mellitus—A population-based study. The HUNT study in Norway	These results suggest a possible role of bromine, cadmium, chromium, iron, nickel, silver and zinc in the development of type 2 diabetes.	doi: 10.1016/j.jtemb.2016.12.008
Association between iron level, glucose impairment and increased DNA damage during pregnancy	Moderate ferritin levels to iron intake without iron-supplement, at early pregnancy is a modifying factor for the correlation of oxidative damage and glucose intolerance in pregnant women.	doi: 10.1016/j.jtemb.2016.11.006
Insulin treatment corrects hepcidin but not YKL-40 levels in persons with type 2 diabetes mellitus matched by body mass index, waist-to-height ratio, C-reactive protein and Creatinine	Levels of hepcidin is important for reducing iron-overload, which is a risk factor for prediabetes.	doi: 10.1186/s12902-017-0204-4
Association of serum ferritin levels with metabolic syndrome and insulin resistance in a Chinese population	Metabolic syndrome prevalence increased with elevated serum ferritin levels	doi: 10.1016/j.jdiacomp.2016.06.018
Serum ferritin level is positively associated with insulin resistance and metabolic syndrome in postmenopausal women: A nationwide population-based study	This study suggest that serum ferritin level in postmenopausal women may help to identify the presence of insulin resistance and metabolic syndrome.	doi: 10.1016/j.maturitas.2017.06.004
Associations between dietary intakes of iron, copper and zinc with risk of type 2 diabetes mellitus: A large population-based prospective cohort study	Dietary intakes of iron and copper were associated with a higher risk of T2DM, while dietary intake of zinc was associated with a reduced risk of T2DM in Japanese population.	doi: 10.1016/j.clnu.2017.02.010
Genome-wide association study of iron traits and relation to diabetes in the Hispanic Community Health Study/Study of Latinos (HCHS/SOL): potential genomic intersection of ironand glucose regulation?	These results provide new genetic information on iron traits and their connection with glucose homeostasis	doi: 10.1093/hmg/ddx082
Association of serum ferritin levels with smoking and lung function in the Korean adult population: analysis of the fourth and fifth Korean National Health and Nutrition Examination Survey	Serum ferritin levels were increased in former or current smokers	doi: https://doi.org/10.2147/COPD.S116982

indicated by the clinical data (Haap, 2003), because if the numerical data prove high affinity between the target-ligand system involved in the biochemical cascade of the disease, is because the iron can contribute to trigger alterations in the glucose metabolism. The data from the *in silico* experiments suggest a molecular view of the problem situation of the present study. Several studies have shown the association between elevated iron stores and insulin resistance in different population groups, including individuals with diabetes (Mainous, 2014), individuals without diabetes (Arija, 2014 and Friedwald, 1972), women with gestational diabetes (Lao, 2001) and patients with thalassemia (Cario, 2003). Also in this sense, type 2 diabetes is common in patients with hereditary hemochromatosis (Mainous, 2014; Orban, 2014; Simcox, 2013; Hansen, 2014), and phlebotomy in these patients results in an improvement in insulin resistance (Andrews, 2005; Hansen, 2014). Finally, prospectives studies have demonstrated the association between high iron stores and the incidence of diabetes (Hansen, 2014; Ramakrishnan, 2002; Salonen, 1998).

The Supplement 1 (S1) lists recent articles confirming the clinical data this study (results section), *insilico* experiments (table 3) and the aspects of metabolism of the Figure 1. The Non-Communicable Diseases (NCDs) are non-infectious and non transmissible diseases that may be caused by genetic or behavioral factors and generally have a slow progression and long duration. These include cardiovascular diseases, cancer, chronic respiratory diseases, and DIABETES. Considering the results obtained in this research, compatible with results of other studies, it was possible to systematize the clinical screening framework in order to intensify prevention with evidence to avoid and / or reverse the negative effects caused by diabetes.

CONCLUSIONS

Considering the investigative approaches used here, this work corroborates the assertion that the elevation in the iron stock is associated with the risk of developing diabetes and / or generate alterations in glucose metabolism. Thus, it is

necessary to verify or even explore the role of elements associated with iron metabolism [40], such as alterations of the HFE gene, DMT1, ferroportin, hepicidin, transferrin receptor, among others, during the development process of type 2 diabetes mellitus, since it has been observed that some genes are involved simultaneously in the balance of organic iron, inflammatory processes and elements responsive to glucose, suggesting a connection between these and the development of type 2 diabetes. Finally, the results of this study aims to contribute to an effective and low-cost public health strategy in the fight against diabetes and its systemic complications.

Ethical Approval and Consent to participate: The procedures performed in this study were approved by the Ethics Committee of the Hospital Universitário Presidente Dutra da Universidade Federal do Maranhão (HUUFMA), under reference number 56943.

Trial registration number: 04151712.0.0000.5086
Name of registry: INADEQUADO CONTROLE DA GLICEMIA EM PACIENTES COM DIABETES TIPO II EM ASSOCIAÇÃO A MARCADORES DE INFLAMAÇÃO SISTÊMICA

URL of Registry: http://plataformabrasil.saude.gov.br/login.jsf
The authors of the article declare that all the individuals , in separate, who participated of the study received appropriate guidance of the objectives of the study and signed the Informed Consent Term (ICT) for participation in this study.

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