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Association between ferritin and hepcidin levels and inflammatory status in patients with type 2 diabetes mellitus and obesity

Mónica Andrews Ph.D. [a], Néstor Soto M.D. [b], Miguel Arredondo-Olguín Ph.D. [a,*]

[a] Micronutrient Laboratory, Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago, Chile
[b] Endocrinology and diabetes Unit, Hospital San Borja Arriarán, Santiago, Chile

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ABSTRACT

Objective: The aim of this study was to determine the association between iron parameters and inflammation in obese individuals with and without type 2 diabetes mellitus (T2DM).
Methods: We studied 132 obese individuals (OB), 60 individuals with T2DM, 106 obese individuals with T2DM (T2DOB), and 146 controls (C). All of were men aged >30 y. Biochemical, iron nutrition, and oxidative stress parameters were determined. Peripheral mononuclear cells were isolated and total RNA was extracted to quantify tumor necrosis factor (TNF)-α, nuclear factor (NF)-κB, interleukin (IL)-6, toll-like receptor (TLR)-2/4 and hepcidin by quantitative reverse transcription polymerase chain reaction.
Results: OB, T2DM, and T2DOB individuals had higher ferritin, retinol-binding protein 4, and thiobarbituric acid reactive substance (TBAR) levels than controls. T2DOB and T2DM individuals showed high high-sensitivity C-reactive protein (hsCRP) levels and OB with and without T2DM had elevated levels of serum hepcidin. Heme oxygenase activity was high in OB and T2DM and there were no differences observed in superoxide dismutase and glutathione parameters. A correlation between TBARS and ferritin in T2DOB was observed ($r = 0.31$; $P < 0.006$). Multiple linear regression analysis showed an association between diabetes and obesity with ferritin, TBARS, and hsCRP levels. The upper quartiles of ferritin, TBARS and hepcidin showed an adjusted odd ratio for T2DM of 1.782, 2.250, and 4.370, respectively. TNF-α, IL-6, hepcidin, NF-κB, TLR-2/4 mRNA abundances were increased in T2DM and T2DOB.
Conclusion: Elevated hsCRP and hepcidin levels, and increased gene expression of TNF-α, IL-6, NF-κB, and TLR-2/4 in patients with diabetes, obesity, or both exacerbate and perpetuate the insulin resistance and inflammatory state.

Introduction

Obesity and type 2 diabetes mellitus (T2DM) present mild chronic inflammation and insulin resistance (IR) in tissues such as adipose, muscle, pancreas, and liver [1,2]. Obesity generally precedes diabetes because visceral adipose tissue has particular cellular and metabolic characteristics. This condition generates IR and hyper-lipolytic effects [3]. Adipose tissue is an endocrine organ that secretes cytokines, adipokines, chemokines, and different growth factors [4–6].

Proinflammatory cytokines play a role in obesity and T2DM development. Immune tissues participate in IR and T2DM development [7,8], where glucose levels and macronutrients (saturated fatty acids) [9], oxidative stress (OS) [10], and micronutrients–specifically iron [9]–are key components. A clear association has been found between obesity, metabolic syndrome, T2DM and the immune system [11]. Toll-like receptors (TLRs) are proteins that participate in the development of inflammatory diseases. TLRs 2 and 4 are involved in IR and T2DM because they

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* Corresponding author. Tel.: +56 29 78 1483; fax: +56 22 21 4030.
E-mail address: marredon@inta.uchile.cl (M. Arredondo-Olguín).

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recognize fatty acids, glucose, advanced glycated end products, reactive oxygen species (ROS), and lipid peroxidation products [12]. Stimulation of TLR-2/4 activates nuclear factor (NF)-κB through nicotinamide adenine dinucleotide phosphate oxidase and monocyte chemoattractant protein-1, which stimulates macrophages, as well as proinflammatory cytokine synthesis [11, 13]. Also, adipocytes infiltrated for macrophages disrupt homeostasis by exacerbation of inflammatory response [14]. The activation of NF-κB by TLR-2/4 in adipocyte and others cellular types trigger the increasing of synthesis of tumor necrosis factor (TNF)-α [15], interleukin (IL)-6, and IL-1 [16–18].

Iron (Fe) has a potential role in diabetes [19], as it is a powerful prooxidant that causes an increase in ROS and OS, contributing directly to tissue damage, raising the risk for diabetes [20]. In addition to being the main marker of iron status, ferritin is an acute-phase protein whose levels increase during inflammatory processes, even in Fe-deficient conditions. Ferritin production is induced in macrophages, hepatocytes, and adipocytes through TNF-α and IL-1 action. High ferritin levels in T2DM have been associated to increased glucose and insulin levels, as well as hypertension, dyslipidemia, and obesity [21]. Hepcidin (Hpc), a 25 amino acid hormone, is synthesized in liver, adipose tissue, pancreas, and intestinal cells. Hpc synthesis is stimulated by iron stores, during infections and inflammations and downregulated during erythropoiesis and hypoxia [18]. Hpc negatively regulates Fe input into the bloodstream through the internalization and subsequent lysosomal degradation of the Fe output conveyor ferroportin (FPN; IREG1) in duodenum and macrophages [22]. Individuals with T2DM possess high levels of Hpc mRNA, and protein, which correlates positively with IL-6 and ferritin levels [23]. IL-6 regulates Hpc synthesis at a transcriptional level through STAT-3, inducing an increase in iron storage levels in macrophages [23].

Both OS and inflammation interact in chronic diseases development such as atherosclerosis, kidney failure, cancer, and T2DM [24]. Heme oxygenase (HO1), an inducible enzyme that catalyzes the degradation of heme to carbon monoxide (CO), biliverdin and Fe^{2+}, regulates cell proliferation, differentiation, and apoptosis as well as attenuates inflammation and modulates the immune response [25]. HO1 possesses binding sites for NF-κB and glucocorticoid response elements, where it might play a role in reducing inflammation during OS and glucose regulation [26].

The aim of this work was to study the association between general nutritional status, OS, Fe nutrition, and inflammation in obese individuals with or without T2DM.

Methods

Study participants

We studied 444 individuals: 106 obese with type 2 diabetes (T2DOB); 132 obese nondiabetic (OB) individuals; 60 (T2DM) patients with T2DM and a normal body mass index (BMI); and 146 healthy individuals (C group). All individuals were men aged >30 y. T2DOB and T2DM patients with insulin treatment were not included in the study. Patients with diabetes were undergoing treatment with metformin. None of the individuals were supplemented with minerals. The protocol was approved by the ethical committee of the Institute of Nutrition and Food Technology, University of Chile and a signed consent form was obtained from all participants.

Anthropometric examination and blood sampling

All patients were weighed, measured, and their BMI was calculated. Also, waist circumference and blood pressure was determined. Blood samples (30 mL) were obtained after overnight fasting. Eighteen mL of blood was used to measure biochemical indicators: glycemia (Dialab, Austria); insulin (radioimmunoassay, Siemens, Los Angeles, CA, USA); lipid profile (Dialab, Austria); high-sensitivity C-reactive protein (hsCRP; Orion Diagnostica, Espoo, Finland), retinol-binding protein 4 (RBP4; by enzyme-linked immunosorbent assay [ELISA]); thiobarbituric acid reactive substances (TBARS; OxiSelect™ TBARS Assay Kit, Cell Biolabs, San Diego, CA, USA); Glutathione (GSH) and total superoxide dismutase (SOD) activity (ELISA kit, Cayman Chemical Com, An Arbor, Michigan, USA). Hematologic and Fe nutrition status such as hemoglobin (Cell Dyn 3200 counter; Abbott Laboratories, Abbott Park, IL, USA), ferritin (measured by using ELISA; Dako Corp, Carpinteria, CA, USA), and Hpc (by ELISA, DRG Instruments GmBH, Germany); Fe and total iron-binding capacity (TIBC; by colorimetric method using tripyridyl-s-triazine [27]); soluble transferrin receptor (TfR; ELISA, Ramco, Stafford, TX, USA). Total body iron (TBI) was calculated according to previous work [28].

Peripheral mononuclear cell isolation and HO1 enzymatic activity

A 12-mL blood sample was collected with EDTA anticoagulant for peripheral mononuclear cell (PMC) isolation. PMCs were separated by Ficoll-Histopaque (1.119 density, Sigma, St. Louis, MO, USA). The mononuclear layer was removed and washed twice in phosphate-buffered saline, adjusted to 40×10^6 PMCs/mL using RPMI-1640 media with gentamicin, and stored until further procedure. HO1 enzymatic activity was measured according to previous work [29].

Quantitative real-time PCR

RNA was extracted from PMCs using Trizol Reagent (Invitrogen) and treated with RNase-Free DNase Set (Qiagen) according to product protocol. Total RNA (1.5 μg) was reverse transcribed using an AffinityScrip cDNA Synthesis Kit (Stratagene). Quantitative polymerase chain reaction (qPCR) was performed using Brilliant II SYBR™ Green QPCR Master Mix (Stratagene) in a Max Pro™ System 3000. Glyceraldehyde 3-phosphate dehydrogenase (*GADPH*) and beta-2-microglobulin (B_2M) were used as housekeeping genes. The primers used were (5′ and 3′, respectively): **GADPH**: CCAGCAAGAGCACAAGAGGA and TCAAGGGGTCTAC ATGGCAA; B_2M: GATGCCGCATTTGGATTGGA and TGGAGCAA CCTGCTCAG ATA; **Hpc**: GACACCAGAGCAAGCTCAA and GAAAACAGAGCCA CTGGTCA; **NF-κB**: TGCATCCAAAGGTGCTCAGA and GCAGCTGGCAAAGCTTAGTA; **IL-6**: ATGTCTGAGGCTCATTCTGC and GCGGCTACATCTTTGGAATC; **TNF-α**: GTTCC TCAGCCTCTTCTCCT and ACAACATGGGCTACAGGCTT; **TLR-2**: AGATGCCTCC CTCTTACCCATGTT and AAGACTTTGGCCAGTGCTTGCT; **TLR-4**: AGGAACAGT GGGTACAGGATGCAA and TCACCCTTAGCATAAGGCCTGACA. PCR amplification efficiency of each primer pair was calculated from the standard curve slope. Final results were reported according to Pfaffl method [30].

Statistical analysis

All variables were checked for normality using Shapiro Wilk test. Anthropometric and biochemical results were expressed as means ± SEM. Anthropometric and biochemical parameter differences between groups were evaluated using one-way analysis of variance and Dunnett's as a post hoc test. Gene expression was analyzed using the Kruskal-Wallis test. For correlation analysis a Pearson's test was performed. STATA 11.0 software was used for linear and logistic regressions. For logistic regression, ferritin and Hpc mRNA expression levels were divided as quartiles. Statistical significance was assigned to $P < 0.05$.

Results

T2DOB and OB showed altered anthropometric parameters such as weight, BMI, and abdominal circumference. T2DM and T2DOB showed increased levels of glycemia and basal insulin, and OB individuals showed higher levels of insulin than C groups. Lipid profiles were similar between groups, except for high-density lipoprotein cholesterol, which remained higher in OB individuals compared with C (Table 1).

OB, T2DM, and T2DOB patients showed increased levels of ferritin. There was no difference in TfR levels. Nevertheless, TBI was higher in OB and T2DOB patients compared with the C group. RBP4 was increased in all studied groups compared with the C group. Hpc protein was elevated in all studied groups and a positive correlation was found between serum Hpc levels and TBI ($r = 0.45$; $P < 0.001$). hsCRP levels were increased mainly in T2DM and T2DOB patients. T2DM and OB patients displayed the highest HO activity and OB, T2DM, and T2DOB groups had higher TBARS levels than C subjects (Table 2). There were no significant

Table 1
Anthropometric and biochemical parameters in study participants

	C* (n = 146)	OB* (n = 132)	T2DM* (n = 60)	T2DOB* (n = 106)
Age (y)	51.4 ± 15.7	51.5 ± 10.6	61.3 ± 10.0†	58.3 ± 8.3†
Weight (kg)	69.9 ± 8.1	92.2 ± 13.9†	71.5 ± 8.3	89.5 ± 9.2†
Body mass index (kg/m^2)	24.6 ± 2.2	32.3 ± 3.5†	25.4 ± 2.1	31.4 ± 2.5†
Abdominal circumference (cm)	89.9 ± 7.1	105.7 ± 9.8†	95.8 ± 7.5‡	108.1 ± 10.2†
Basal glycemia (mg/dL)	92.8 ± 11.5	101.3 ± 22.6	185.3 ± 94.7†	183.1 ± 72.1†
Basal insulin (ng/mL)	5.4 ± 4.0	13.4 ± 17.2§	15.2 ± 23.2†	21.5 ± 22.7†
Total cholesterol (mg/dL)	190.3 ± 41.0	190.6 ± 37.7	193.1 ± 63.3	197.4 ± 56.2
High-density lipoprotein cholesterol (mg/dL)	38.0 ± 11.9	32.9 ± 8.2†	36.3 ± 12.2	34.9 ± 11.5
Low-density lipoprotein cholesterol (mg/dL)	122.5 ± 34.2	119.6 ± 33.0	118.1 ± 41.1	115.0 ± 40.5
Triglycerides (mg/dL)‖	131.5 (80.0–216.3)	167.9 (97.9–288.2)	139.8 (73.1–267.1)	199.0† (106–374)

ANOVA, analysis of variance
* Values are mean ± SD.
† One-way ANOVA, post hoc Dunnett's $P < 0.001$.
‡ One-way ANOVA, post hoc Dunnett's $P < 0.01$.
§ One-way ANOVA, post hoc Dunnett's $P < 0.05$.
‖ Values are geometric mean ± (range).

differences in SOD and GSH activity between groups (data not shown).

To determine whether ferritin levels were a risk factor for T2DM development, we performed a multiple linear regression analysis adjusted by age. T2D was independently associated with increased ferritin ($P = 0.001$), TBARS ($P < 0.001$), and hsCRP ($P = 0.008$) levels. In the presence of obesity, this association increased for ferritin ($P < 0.001$) and TBARS ($P < 0.0001$). Obesity alone was not associated with increased TBARS or hsCRP levels ($P > 0.05$), but showed association with increased ferritin levels ($P = 0.001$). In T2DOB patients, ferritin levels were correlated with serum TBARS ($r = 0.36$; $P < 0.006$). By studying the risk for development of T2DM in an age-adjusted model, BMI and hsCRP, between upper and lower quartiles of ferritin and TBARS levels, we found a high odds risk in the upper ferritin quartile. Also, increased TBARS levels showed an elevated odds risk for development of T2DM (Table 3). When participant distribution was analyzed according to Hpc mRNA fold-change expression, we observed that the highest risk for T2DM development was in the fourth quartile with or without BMI and hsCRP adjustments (Table 4).

Hpc, TNF-α, NF-κB, and TLR-4 mRNA relative abundances were increased in OB, T2DOB, and T2DM patients (Fig. 1A, C, E, and F, respectively). Also, the expression of IL-6 and TLR-2 were increased in T2DOB and OB groups (Fig. 1B and D).

Discussion

The association between Fe stores and inflammation in obese individuals with and without diabetes, as well as in diabetic patients with normal nutritional status was studied. These patients were found to have higher ferritin levels than those in the control arm. Increased levels of hsCRP in individuals with diabetes with and without obesity also were observed. Ferritin, a protein that reflects body iron stores, is also an acute-phase protein that is elevated in inflammatory conditions [31,32]. In our study, it was not possible to determine whether the increased values of ferritin were due to an overload of Fe or a consequence of the underlying proinflammatory profile. However, similar observations have been shown in other studies, where high levels of ferritin have been suggested to be one of many components of the IR syndrome [32], and have also been correlated with the onset of diabetes [24]. It had previously been postulated that if high ferritin levels reflect high circulating Fe concentrations, this could alter hepatic insulin clearance, resulting in hyperinsulinemia; induce a decreased insulin secretion in the pancreas [33]; and induce β-cell apoptosis [34].

An increase in TBARS was found, reflecting increased lipoperoxidation in OB, T2DM, and T2DOB patients. Both the T2DM and OB groups showed high HO activity, suggesting that

Table 2
Iron nutrition and oxidative stress parameters in study participants

	C* (n = 146)	OB* (n = 132)	T2DM* (n = 60)	T2DOB* (n = 106)
Hemoglobin (g/dL)	15.7 ± 1.3	16.2 ± 1.2†	14.7 ± 1.8‡	15.3 ± 1.6
Serum ferritin (μg/L)‖	56.5 (33.7–90.9)	75.5‡ (50.0–111.0)	70.3§ (42.2–118.1)	82.3‡ (53.9–125.7)
Serum iron (μg/dL)	107.5 ± 37.4	100.2 ± 35.1	124.2 ± 88.2	107.9 ± 50.7
Transferrin saturation (%)	32.7 ± 12.0	30.5 ± 9.0	35.6 ± 15.9	27.9 ± 9.7†
Transferrin receptor (μg/mL)‖	2.8 (1.2–6.4)	3.9 (1.3–6.6)	3.6 (1.8–7.4)	3.6 (1.9–6.5)
TBI (mg/kg)	8.5 ± 3.2	9.7 ± 3.3†	9.1 ± 3.7	9.6 ± 2.7†
Hepcidin (ng/mL)	19.0 ± 8.7	25.0 ± 11.5†	23.4 ± 10.6†	25.2 ± 10.8§
RBP4 (μg/mL)	26.1 ± 8.4	33.6 ± 7.3†	31.7 ± 9.6†	32.7 ± 9.3†
hsCRP (μg/dL)‖	0.8 (0.2–4.4)	1.8 (0.5–6.8)	1.9§ (0.4–7.8)	2.0§ (0.4–9.0)
HO1 (nmole bilirubin·mg protein·h)‖	2.6 (0.9–7.1)	4.2† (1.7–10.1)	4.6‡ (1.7–12.4)	3.4 (0.4–9.0)
TBARS (nmoles/mL)‖	0.99 (0.4–2.4)	1.4† (0.7–2.7)	1.7§ (1.0–3.1)	2.1‡ (1.2–3.5)

ANOVA, analysis of variance; HO1, heme oxygenase-1; hsCRP, high-sensitivity C-reactive protein; RBP4, retinol-binding protein 4; TBARS, thiobarbituric acid reactive substances; TBI, total-body iron
* Values are mean ± SD.
† One-way ANOVA, post hoc Dunnett's $P < 0.05$.
‡ One-way ANOVA, post hoc Dunnett's $P < 0.001$.
§ One-way ANOVA, post hoc Dunnett's $P < 0.01$.
‖ Values are geometric mean ± (range).

Table 3
Risk for developing type 2 diabetes (OR) according to ferritin quartiles and TBARS concentration

	OR* without to adjust	CI	*P*-value	OR adjusted†	CI	*P*-value
Ferritin Q1: <50 μg/L	1.000			1.000		
Ferritin Q2: 50–100 μg/L	1.021	0.42–1.71	0.12	1.101	0.87–1.57	0.66
Ferritin Q3: 100–150 μg/L	1.302	0.45–1.91	0.09	1.133	0.66–1.83	0.08
Ferritin Q4: 150–200 μg/L	1.377	0.97–2.19	0.07	1.782	1.61–1.92	<0.01
TBARS	1.980	1.91–2.28	<0.05	2.250	1.89–3.25	<0.05

TBARS, thiobarbituric acid reactive substances
* OR were estimated through logistic regression.
† Adjusted to age, body mass index, and high-sensitivity C-reactive protein.

these individuals are under OS conditions. Also, we observed a positive correlation between serum TBARS and ferritin in the T2DOB group. Increases in TBARS and other OS markers have been observed previously [25], showing that in T2DM there is increased activation of pathways that generate OS. HO1, a protein induced under stress conditions, is capable of counteracting OS, acting as a protection system against cellular stress [34]. Increased HO activity in hyperglycemic rats has been associated to a decrease in superoxide production [35]. In T2DM and OB patients two main facts were observed: 1) different levels of OS and 2) increased HO activity. However, T2DOB participants showed a slight increase of this enzymatic system with an increase in TBARS, demonstrating that this group is under high OS. It was also observed that obesity and diabetes have a strong relationship with increased ferritin and TBARS levels. This association in individuals with obesity but with no diabetes was observed only throughout ferritin levels. Nevertheless, and independent of the nutritional status, T2DM showed a close relationship with increased inflammation (hsCRP), nutritional iron markers (ferritin and Hpc) and OS (TBARS). Additionally, high ferritin and TBARS levels are risk factors for development of T2DM. Similar results were observed in the Nurse Cohort study in the highest quartile of ferritin, yielding an odds ratio of 2.68 [36]. Notably, no Fe overload was observed in our study group (ferritin levels >300 mg/L); demonstrating that a small increase in ferritin levels—and therefore Fe deposit levels—constitute a risk factor for development of T2DM.

RBP4, the main retinol (vitamin A) transporter protein, is secreted by adipose tissue and the liver [37]. RBP4 increases in the serum of obese individuals, IR, and T2DM [38,39] and is negatively associated with adiposity and inflammatory parameters [40]. The association between RBP4 and visceral fat is stronger than with BMI, indicating a primary role for visceral fat rather than subcutaneous fat [41]. Glut4 expression in human adipocytes is positively correlated with the deposition rate of glucose and inversely with serum levels of RBP4 [38], therefore, the link between RBP4 and T2DM would be through a decreased insulin secretion. The pattern of serum RBP4 concentration resembles Fe deposits: higher in men than in women. The interaction between Fe and vitamin A is known. Vitamin A deficiency alters Fe metabolism and exacerbates anemia. There is a direct association between the levels of Fe and vitamin A. Fe supplementation increases vitamin A and also RBP4 [42]. Serum ferritin had been positively associated with RBP4 levels [42] and serum RBP4 levels decrease after Fe depletion in T2DM individuals. In summary, there are clinical and experimental evidence that suggest an association between RBP4 and obesity, IR, and Fe metabolism.

We evaluated relative abundance of genes associated with inflammation and Fe metabolism. We observed that *NF-κB* and *TNF-α* relative abundances were increased in OB, T2DM, and T2DOB. One study [14] demonstrated that adipocytes expressed *TNF-α*, and that this expression was markedly elevated in ob/ob, db/db, and fa/fa animals with diabetes and/or IR, respectively. Adipose tissue, particularly visceral, expresses and secretes other cytokines such as IL-6 and IL-1, and therefore obese individuals showed increased levels of these proteins [9]. Increased levels of IL-6 expression were observed in T2DOB and T2DM patients. These results may suggest that the inflammation observed in T2DM is independent of adiposity. Both TNF-α and IL-6 may induce IR in liver and muscle tissue [43], and among the stimuli that induce cytokines expression in these patients, are increased OS and increased levels of circulating glucose and lipid peroxidation [11,44]. We observed high NF-κB mRNA abundance in OB, T2DM, and T2DOB patients, probably because of the high circulating glucose levels and increased OS in those with T2DOB and T2DM. Therefore, increased TNF-α and IL-6 in response to NF-κB, may lie in the increased OS in T2DM and T2DOB and reduced HO activity observed in the T2DOB group. TLR-2/4 are components of the immune system that are activated by nutrients such as glucose [45] and saturated fatty acids [11], and also for advanced glycation species and lipid peroxidation products [12,46]. In PMCs from T2D and T2DOB individuals, we observed increased TLR-2/4 expressions. It has been shown that TLR-2/4 expressions are increased in different cell types in patients with atherosclerosis and obesity [47]. TLR receptor activation induces NF-κB activation and therefore the amplification of inflammatory response through the induction of IL-6 and IL-1β, leading to the exacerbation of IR and diabetes [48].

Hpc is the major hormone that regulates circulating Fe and is also an acute-phase protein. The main stimulus for Hpc expression and secretion is inflammation (IL-6-, IL-1β- and LPS-dependent) and intracellular Fe levels [49]. We found that OB patients with and without T2DM had elevated levels of serum Hpc, as well as ferritin and Hpc mRNA and the OB groups had elevated levels of hsCRP. In obesity, the increased visceral adipose tissue contributes to increase circulating Hpc levels [50]. High-circulating Hpc levels have been observed in overweight children and overweight individuals have been found to have lower Fe status measured as Fe-dependent erythropoiesis [51].

Table 4
Risk for developing type 2 diabetes (OR) according to hepcidin expression quartiles

	OR*	CI	*P*-value	OR adjusted†	CI	*P*-value
Hepcidin Q1	1.000			1.000		
Hepcidin Q2	1.483	1.11–1.98	0.007	1.300	0.95–1.11	0.470
Hepcidin Q3	1.980	0.89–4.37	0.091	2.120	0.89–5.03	0.087
Hepcidin Q4	3.291	1.39–7.75	0.006	4.370	1.67–11.42	0.003

* OR were estimated through logistic regression.
† Adjusted to age, body mass index, and high-sensitivity C-reactive protein.

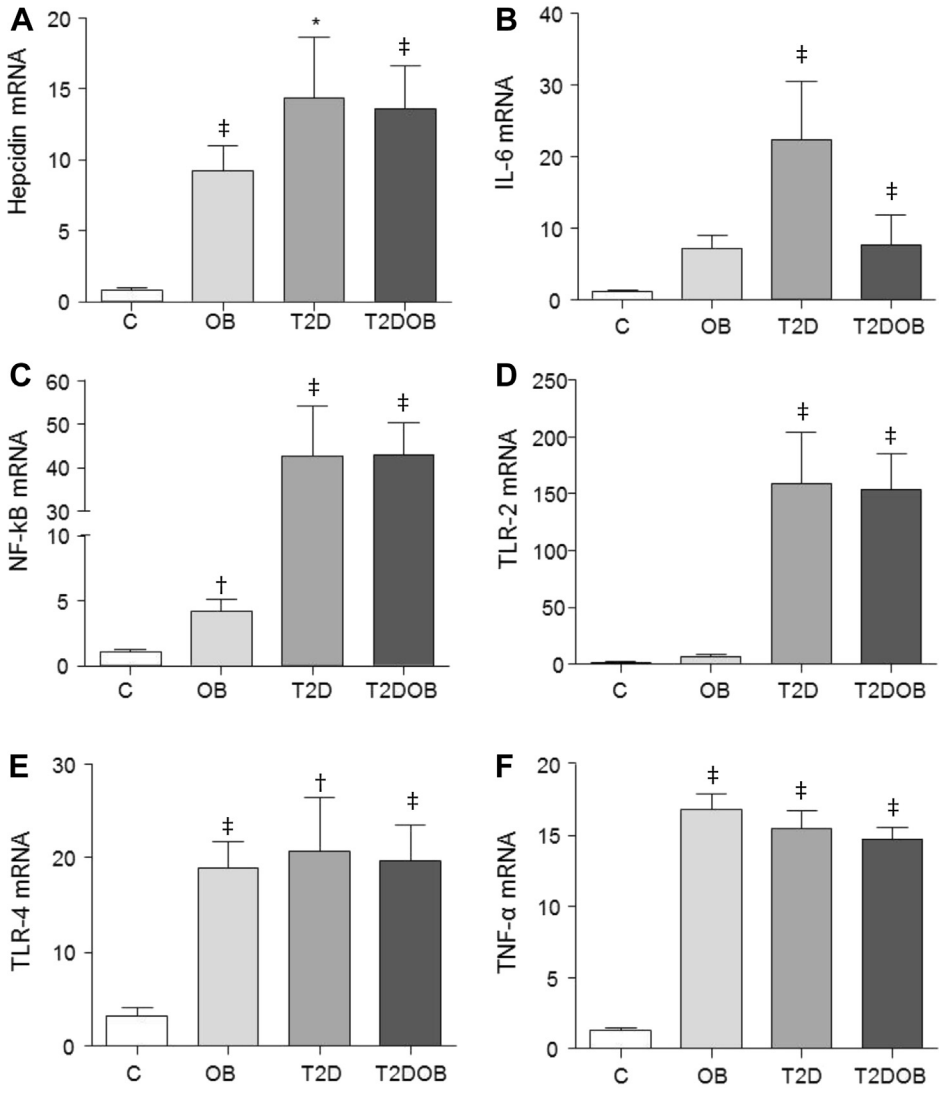

Fig. 1. Relative abundance of genes related to inflammation in OB, T2DOB, T2DM, and C participants. (A) hepcidin; (B) IL-6; (C) NF-κB; (D) TLR-2; (E) TLR-4; (F) TNF-α. Values are mean ± SEM. Data were analyzed using the Kruskal-Wallis test. $^{*}P < 0.05$. $^{\dagger}P < 0.01$. $^{\ddagger}P < 0.001$. C, control; IL, interleukin; NF, nuclear factor; OB, obese; T2DM, type 2 diabetes mellitus; T2DOB, type 2 diabetes mellitus obese; TLR, Toll-like receptor; TNF, tumor necrosis factor.

Hpc is also related to diabetes, as was shown in a study of 30 women with gestational diabetes who had elevated Hpc levels compared with control and with impaired glucose tolerance [52]. The women with gestational diabetes also had increased serum ferritin and Fe, however, none of the Fe parameters was correlated with inflammatory markers. We found an increased Hpc mRNA expression compared with the C group. It has been shown that monocytes express and synthesize Hpc; however, its exact function has not yet been determined. These findings suggest that Hpc from PMCs play a paracrine role in Fe regulation under a proinflammatory environment [53]. In this study, we showed that the inflammation in these patients increased Hpc expression in PMCs, results that may explain the changes observed in body Fe distribution in OB, T2DOB, and T2DM individuals. Additionally, we showed that the highest Hpc expression levels were a risk factor for development of T2DM. IL-6 binding to its receptor results in the activation of STAT-3, which is translocated to the nucleus, and interacts with IL-6 response elements in the Hpc promoter [11]. We observed increased IL-6 expression, probably mediated by Hpc stimulus in the PMCs of T2DOB and T2DM patients.

In summary, we demonstrated that individuals with obesity and diabetes have high hsCRP, and high Hpc, TNF-α, IL-6, NF-κB, *TLR-2/4* gene expression, which exacerbate and perpetuate inflammation and IR. We showed that diabetes, regardless of the nutritional status of the patients, is associated with altered body Fe distribution, observed in high levels of ferritin and TBI found in participants with T2DM regardless of obesity. Obesity usually precedes T2DM, and is associated with elevated levels of ferritin. Disturbances in Fe status in addition to increased OS levels, plays a pivotal role as a risk factor for T2DM development, but also in the onset of complications of this disease (Fig. 2). We showed that increased ferritin and TBARS level, and elevated Hpc expression increased the risk for T2DM development. Nevertheless, inflammation in patients with T2DM was independent of adiposity; therefore, the origin of inflammation could be due to

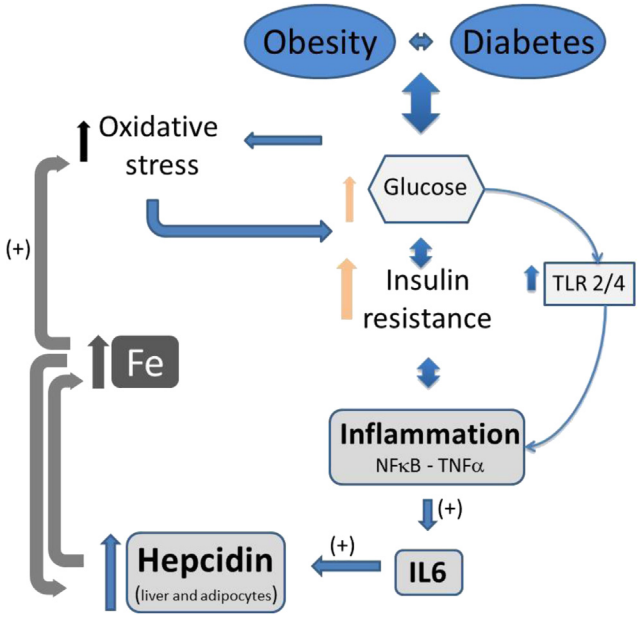

Fig. 2. Schematic diagram of interactions between metabolic alterations in diabetes/obesity and inflammatory stimuli and hepcidin. Hyperglycemia in obesity/diabetes induces the activation of TLR-2/4, which triggers the inflammation cascade that includes NF-κB and TNF-α, producing insulin resistance. At the same time, hyperglycemia generates oxidative stress, which exacerbates the insulin resistance. NF-κB induces IL-6 expression. IL-6 is an inducer of hepcidin in liver and adipocytes cells. High levels of iron (plasmatic iron or storage iron as ferritin) participate actively in oxidative stress; iron is also a hepcidin inducer. Elevated expression of hepcidin increases the internalization and degradation of ferroportin (iron exporter) in enterocytes and monocytes and as result there is an increase in intracellular iron levels and oxidative stress. IL, interleukin; NF, nuclear factor; TLR, Toll-like receptor; TNF, tumor necrosis factor.

increased levels of glucose and/or OS found in the group with diabetes.

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