Iron metabolism in type 1 diabetes: relation to insulin resistance

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Background
An evidence-based association was established between iron metabolism and insulin-resistant (IR) conditions, among which was type 2 diabetes. Previous studies have reported elevated hepcidin and ferritin levels in type 2 diabetics.
Aim
The aim of this study was to investigate the possible relationship between hepcidin or ferritin and the development of IR in type 1 diabetes mellitus (T1DM).
Methodology
The study included 60 male participants who were categorized as follows: 20 patients having T1DM with IR (group 1), 20 patients having TIDM without IR (group 2), and 20 age-matched and BMI-matched healthy individuals. IR was evaluated using estimated glucose disposal rate (eGDR) and insulin (U/day). All patients were tested for fasting blood sugar, postprandial blood sugar, hemoglobin A1c, lipid profile, high-sensitivity C-reactive protein, C-peptide, ferritin, and hepcidin.
Results
Serum hepcidin showed a nonsignificant difference between groups 1 and 2, and was not correlated to any IR-related variables. Serum ferritin was significantly higher in group 1, positively correlated to BMI, waist circumference, insulin (U/kg/day), and negatively correlated to eGDR. Out of all the significantly correlated variables, the hemoglobin A1c and waist/hip ratio were able to predict eGDR using the multivariate analysis.
Conclusion
Hepcidin plays no role in T1DM IR patients. Although ferritin was higher in T1DM patients and was negatively correlated to eGDR, it failed to demonstrate an independent influence on eGDR, hindering its potential use as a predictor of IR.

Keywords:
diabetes mellitus, estimated glucose disposal rate, ferritin, hepcidin, insulin resistance, type 1

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Introduction

A number of components of metabolic syndrome (MetS) may be observed in patients with type 1 diabetes mellitus (T1DM) and potentially contribute to increased cardiovascular (CV) risk [1,2]. In the past, it was thought that insulin resistance (IR) in T1DM was primarily related to hyperglycemia [3,4]. However, in recent years, new data have emerged that challenge this concept. Impaired glucose utilization, together with impaired insulin-induced nonesterified fatty acid suppression, exists in T1DM independent of glycemic control. Furthermore, these adults have exhibited IR in hepatic and skeletal muscle tissue despite good glycemic control [5].

A bulk of evidence has established a link between iron metabolism and IR states such as type 2 diabetes mellitus (T2DM) and MetS [1]. The development of T2DM has been positively correlated to baseline ferritin levels, which is the best marker of the body's iron stores. Although it has been hypothesized that elevated transferrin saturation is associated with an increased risk of all forms of diabetes, including types 1 and 2 [3], the complex pathophysiological links between iron and metabolic derangements remain poorly understood [1]. In the past 10 years, hepcidin has emerged as the key iron regulatory hormone. This defensin-like, 25-amino-acid peptide is produced by the liver primarily in response to increased plasma or tissue iron to homeostatically downregulate absorption and recycling of the metal. At the molecular level, hepcidin acts by binding and inactivating its cell membrane receptor, ferroportin, the only known cellular iron exporter. Ferroportin is expressed by cells that are critical for iron homeostasis, such as absorbing duodenal enterocytes, reticuloendothelial macrophages (involved in iron storage and recycling), and hepatocytes (involved in iron storage and endocrine regulation). Hepcidin is also upregulated by inflammatory cytokines, a response

believed to contribute to host defense by subtracting iron from invading pathogens [1].

It is hypothesized that circulating prohepcidin and IR associated with iron overload might be pathophysiologically linked [2]. Hepcidin tends to be high in patients with MetS and IR. Similarly, increased levels of hepcidin and hepcidin gene expression were exhibited in T2DM patients and patients with MetS with or without diabetes. One study showed an increased trend for risk of T2DM in patients with the highest hepcidin gene expression [6]. Hepcidin represents an appealing candidate to be investigated in patients with MetS features or IR features. In view of the rapidly growing evidence of the pleiotropic effects of hepcidin, this may have relevant implications for MetS pathophysiology [1].

To our knowledge, there is only one study that has assessed hepcidin levels in patients having T1DM, in addition to T2DM [7]. T1DM were specifically included in that study to help investigators to adequately interpret variations in hepcidin levels in T2DM and whether they were due to IR or insulin deficiency. Besides, the authors have not distinctively explored hepcidin levels in IR associated with T1DM.

The primary outcome of our study is to determine whether hepcidin and ferritin incur any effect on IR in T1DM patients, whereas the secondary outcome is to detect predictors of IR in T1DM patients.

Methodology

This is a case–control study that has been conducted on T1DM patients. The study was conducted during the period from March 2013 to July 2014, and was approved by the local research ethical committee. All patients were regular attendees of the adult T1DM clinic. Only male participants were included in the study, as antecedent data showed substantial sex differences regarding serum hepcidin levels, with females showing significantly lower values during fertile periods [8]. In addition, women generally exhibit a higher incidence of iron deficiency anemia [9,10].

Patients receiving iron supplementation, as well as patients with diabetic nephropathy, chronic kidney disease, infections, and malignancy, were excluded from the study.

All male patients were screened for IR using estimated glucose disposal rate (eGDR). Patients willing to participate in the study were recruited and categorized accordingly into a case group (T1DM with IR, n=20) and a control group (T1DM without IR, n=20). In addition, an equal number of age-matched and BMI-matched healthy male control individuals were selected from workers and employees at the hospital (n=20). All data concerning each patient's diabetes history were retrieved from the clinic files. All patients were given physical examinations to measure anthropometric data, including BMI, waist circumference and waist/hip ratio, and blood pressure. The eGDR was determined for each patient according to the following equation: eGDR (mg/kg/min) = 21.158 + [−0.09×waist circumference +(−3.407×hypertension)+(−0.551×HbA1c)], with a cutoff value of eGDR equal to 7.5 mg/kg/min [11]. In addition, insulin (U/kg/day) was calculated, and fasting blood sugar, postprandial blood sugar, hemoglobin A1c (HbA1c%), lipid profile, high-sensitivity C-reactive protein (hsCRP), C-peptide, ferritin, and hepcidin were measured.

Laboratory assessment

Fasting venous blood samples were collected and separated by centrifugation at 2000g for 15 min at 4°C, and the aliquots were stored at −70°C within 1 h of collection. Serum levels of hepcidin were estimated using a DRG hepcidin-25 bioactive enzyme-linked immunosorbent assay kit (DRG Instruments GmbH, Marburg, Germany) according to the manufacturer's protocol. Intra-assay precision was 5.1%; interassay precision was 12.7%; and analytical sensitivity was 0.35 ng/ml. hsCRP was estimated from sera using an enzyme-linked immunosorbent assay kit (R&D Systems Inc., Minneapolis, Minnesota, USA). Intra-assay precision was 4.1–6.9%; interassay precision was 5.8–6.3%; and analytical sensitivity was 0.02 μg/ml.

Total cholesterol and high-density lipoprotein-cholesterol (HDL-C), glucose, and triglycerides (TGs) were determined from serum samples using the DxC 600 Synchron clinical system (Beckman Coulter Inc., Brea, California, USA). HbA1c was determined from whole blood using the UniCel DxC 600 clinical system (Beckman Coulter Inc.). Serum ferritin and C-peptide were estimated using the Access 2 immunoassay system (Beckman Coulter Inc.).

Low-density lipoprotein cholesterol (LDL-C) was mathematically calculated using the Friedewald equation:

$$\mathrm{LDL-C} = \mathrm{TC} - \mathrm{HDL-C} - \frac{\mathrm{TG}}{5}.$$

If TG was above 400 mg/dl, direct measurement of LDL-C was performed after sequential ultracentrifugation.

Statistical analysis

All data were tabulated and imported to SPSS, version 21 (SPSS Inc.; IBM Corp. Released 2012; IBM SPSS Statistics for Windows, Version 21.0; Armonk, NY: IBM Corp.). Parametric data were represented as mean ±SD, whereas nonparametric data were represented as median and interquartile range. Differences between groups were detected using one-way analysis of variance and post-hoc analysis for parametric data using least significant difference, and the Kruskal–Wallis *H*-test and Mann–Whitney *U*-test were used for nonparametric data. The relationship between hepcidin and ferritin with studied variables was detected using the Spearman correlation coefficient, whereas stepwise multiple linear regression analysis was used to detect predictors for eGDR and HbA1c% among the studied variables.

Results

From 60 male participants among attendees of adult T1DM clinic, 40 patients willing to participate in the study were recruited; female patients were excluded from the study according to the eligibility criteria of the study. They were categorized into group 1 (T1DM with IR, *n*=20), group 2 (T1DM without IR, *n*=20), and group 3 (healthy control individuals, *n*=20).

The mean age for group 1 was 26.5±2.87; the mean age for group 2 was 22.6±2.03; and the mean age for group 3 was 24.5±2.91. Demographic and laboratory data are presented in Table 1. Remarkably, serum hepcidin median and IQR were comparable between groups, showing no significant difference (*P*=0.482).

A comparison between group 1 (T1DM with IR) and group 2 (T1DM without IR) showed a highly significant difference across all variables, with the exception of systolic blood pressure, diastolic blood pressure, LDL-C, HDL-C, and hepcidin, in which there was no significant difference (Tables 2 and 3). A comparison of group 2 and the control group showed a highly significant difference regarding eGDR, fasting blood sugar, postprandial blood sugar, HbA1c%, HDL-C, hsCRP, C-peptide, and ferritin and a nonsignificant difference regarding the rest of the parameters, including hepcidin. Hepcidin showed no correlation with any of the studied variables, whereas ferritin showed a significant positive correlation to BMI, waist circumference, insulin (U/kg/day), and a highly significant negative correlation to eGDR (Fig. 1).

Linear stepwise regression analysis was conducted to detect predictors for eGDR and HbA1c%. Only waist/hip ratio and HbA1c% emerged as predictors for eGDR, whereas waist/hip ratio and eGDR emerged as predictors for HbA1c% (Figs 2 and 3).

Table 1 Demographic and laboratory data of the different study groups

Variables	T1DM with IR (*n*=20)	T1DM without IR (*n*=20)	Control (*n*=20)	*P* value
Age (years)	26.5±2.87	22.6±2.03	24.5±2.91	<0.001
BMI (kg/m^2)	25.43±2.11	22.96±1.02	24.16±1.7	0.001
Waist circumference	92.7±10.2	74±4.9	80.74±8.5	<0.001
W/H ratio	0.92±0.01	0.88±0.01	0.89±0.01	<0.001
SBP (mmHg)	123.5±4.8	119.5±7.5	122.6±9.33	0.09
DBP (mmHg)	77±5.7	76.5±5.8	75.7±5.07	0.83
Insulin (U/kg/day)	1.19±0.08	0.93±0.07	–	<0.001
eGDR (mg/kg/min)	7.14±0.36	9.08±0.41	10.68±0.38	<0.001
FBS (mg/dl)	222±24.9	179.1±23.8	87.26±5.8	<0.001
PPBS (mg/dl)	285.4±22.2	215.45±28.7	103.79±3.02	<0.001
HbA1c%	10.25±0.6	7.8±0.69	4.7±0.4	<0.001
TC (mg/dl)	199.1±26	180.45±14.3	184.4±14	0.01
TG (mg/dl)	202.85±45.6	169.45±15.7	144.32±11.35	<0.001
HDL-C (mg/dl)	42.1±3.8	43.5±3.7	48.8±3.33	<0.001
LDL-C (mg/dl)	150.1±23.6	137.8±13.8	145.7±13.3	0.156
hsCRP (mg/dl)[a]	6865±857.3	4585±980	1831.5±807.6	<0.001
C-peptide (ng/ml)[a]	0.15 (0.1–0.2)	0.4±0.12	1.2±0.3	<0.001
Ferritin (μg/l)[a]	90 (55–200)	55 (36–71)	50 (35–72)	<0.001
Hepcidin (ng/ml)[a]	25 (12–35)	25 (12–37)	15 (7–25)	0.48

DBP, diastolic blood pressure; eGDR, estimated glucose disposal rate; FBS, fasting blood sugar; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; IR, insulin resistance; LDL-C, low-density lipoprotein cholesterol; PPBS, postprandial blood sugar; SBP, systolic blood pressure; T1DM, type 1 diabetes mellitus; TC, total cholesterol; TG, triglyceride; W/H ratio, waist-to-hip ratio. [a]Kruskal–Wallis *H*-test.

Discussion

IR has been recognized as an important component of vascular complications in T1DM, with 1–2% of young adults with T1DM developing coronary artery disease annually [12]. Earlier studies, including The Diabetes Control and Complications trial (DCCT), showed that low levels of eGDR, which is a surrogate for IR in T1DM, predict the development of peripheral artery disease, retinopathy, nephropathy, and coronary artery disease in patients with T1DM, even after 9 years of follow-up. A more recent study showed that eGDR association with chronic complications was stronger than the more established risk factors [13]. Moreover, in some studies, IR in T1DM was demonstrated to be an independent risk factor for developing T1DM alongside other known risk factors. T1DM patients with IR might even show a lower frequency of entering the 'honeymoon phase' [14].

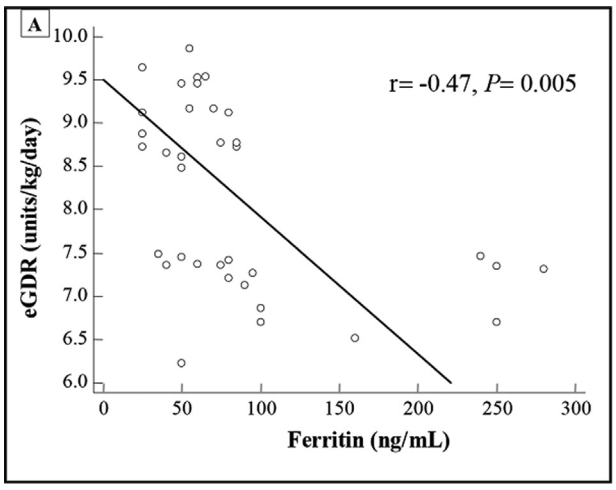
A high prevalence of MetS in T1DM diabetic patients has been reported (38% in men and 40% in women). Pathogenesis of IR in T1DM is still poorly understood, with several hypotheses existing. The concept that IR in T1DM is solely related to adiposity and high HbA1c % has been challenged by recent studies that have demonstrated that the presence of IR in individuals with glycemic control improved to a great extent from the pre-DCCT era (7.5±0.9 and 8.6±1.6% in adults and adolescents) and with BMI similar to that of nondiabetics [12].

Table 2 Comparison between type 1 diabetes mellitus group with insulin resistance and without insulin resistance using post-hoc analysisleast significant difference

	Mean difference	95% CI	*P* value
Age	3.9	1.8–6	<0.001
BMI	2.5	1.18–3.8	<0.001
Waist circumference	18.8	12.4–25	<0.001
W/H ratio	0.041	0.02–0.05	<0.001
SBP	4	–1.78–9.78	0.27
DBP	–0.5	–3.8–4.8	1
Insulin (U/kg/day)	0.26	0.21–0.31	<0.001
eGDR	–1.9	–2.2 to –1.6	<0.001
FBS	43	25.5–60.3	<0.001
PPBS	70	51.6–88.2	<0.001
HBA1c%	2.5	1.9–3	<0.001
TC	18.6	2.2–35	0.01
TG	33.4	6.6–60.1	0.007
LDL-C	12.27	–2.9–27.5	0.2
HDL-C	–1.4	–4.3–1.5	1

CI, confidence interval; DBP, diastolic blood pressure; eGDR, estimated glucose disposal rate; FBS, fasting blood sugar; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PPBS, postprandial blood sugar; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; W/H, waist-to-hip ratio.

Figure 1



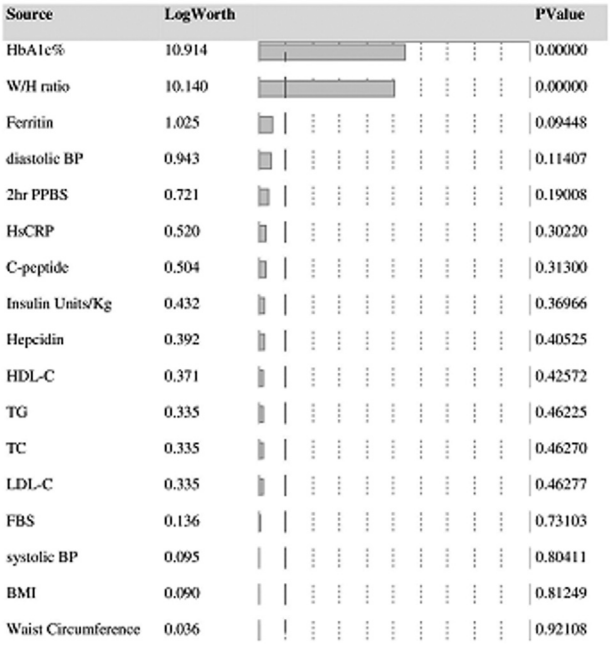
Correlation of ferritin with estimated glucose disposal rate (eGDR).

Table 3 Comparison between type 1 diabetes mellitus group with insulin resistance and without insulin resistance using the Mann–Whitney *U*-test

	Hepcidin	Ferritin	hsCRP	C-peptide
95% CI	18.5–26.8	59.7–91.3	3721.1–4985.8	0.49–0.76
Z value	–0.03	–2.8	–5.4	–4.2
P value	0.973	0.004	<0.001	<0.001

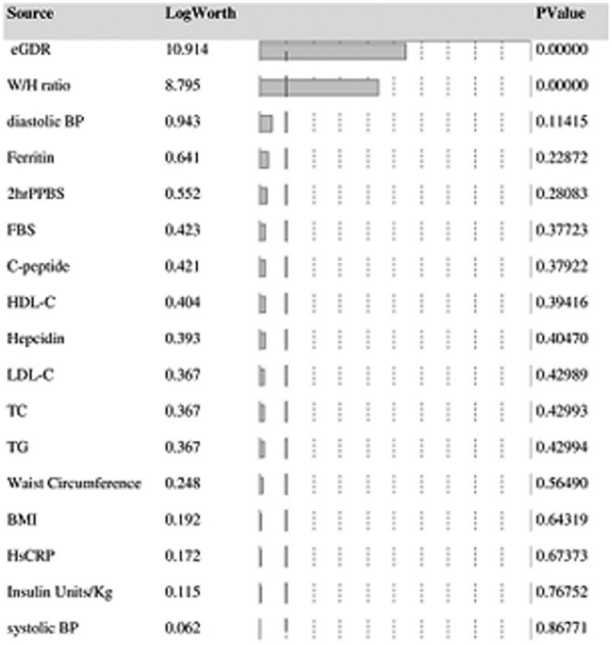
CI, confidence interval; hsCRP, high-sensitivity C-reactive protein.

Figure 2



Linear stepwise regression analysis to detect predictors of insulin resistance among the study population. BP, blood pressure; FBS, fasting blood sugar; HbA1c, hemoglobin A1c; HDL-C,high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; PPBS, postprandial blood sugar; TC, total cholesterol; TG, triglyceride; W/H ratio, waist-to-hip ratio.

Figure 3



Source	LogWorth	PValue
eGDR	10.914	0.00000
W/H ratio	8.795	0.00000
diastolic BP	0.943	0.11415
Ferritin	0.641	0.22872
2hrPPBS	0.552	0.28083
FBS	0.423	0.37723
C-peptide	0.421	0.37922
HDL-C	0.404	0.39416
Hepcidin	0.393	0.40470
LDL-C	0.367	0.42989
TC	0.367	0.42993
TG	0.367	0.42994
Waist Circumference	0.248	0.56490
BMI	0.192	0.64319
HsCRP	0.172	0.67373
Insulin Units/Kg	0.115	0.76752
systolic BP	0.062	0.86771

Linear stepwise regression analysis to detect predictors of metabolic control among the study population. BP, blood pressure; eGDR, estimated glucose disposal rate; FBS, fasting blood sugar; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; PPBS, postprandial blood sugar; TC, total cholesterol; TG, triglyceride; W/H ratio, waist-to-hip ratio.

Data regarding the role of iron metabolism in the pathogenesis of IR in T2DM suggest an influence on glucose metabolism even in the absence of iron overload and below levels of known iron overload syndromes [7,15]. Hepcidin is the key hormone regulating iron homeostasis that has been demonstrated in T2DM to be significantly lower than weight-matched controls and has been negatively correlated with HOMA-IR in recent studies [7].

No previous studies have explored the relation of iron metabolism to IR in T1DM. In the current study, serum hepcidin and ferritin were studied in relation to IR, as was indicated by eGDR and insulin (U/kg), in T1DM patients who were classified with IR and without IR and compared with healthy control individuals. Hepcidin levels showed no difference across both T1DM diabetic groups or when compared with the control group; also, it showed no correlation with any of the studied variables. Nevertheless, serum ferritin differed significantly between T1DM groups and also when the non-IR group was compared with the healthy control group. However, ferritin lacked its significance in determining IR when multivariate analysis was performed to define predictors of IR, debating the effect of ferritin in acquiring IR.

Concordant with our results, Sam *et al.* [7] in his study, which primarily aimed to explore the relationship between hepcidin levels and IR in T2DM, found that there was no significant difference in hepcidin levels between participants with T1DM and control participants, whereas participants with T2DM had significantly lower hepcidin levels than control participants.

Several studies have documented the relationship of ferritin level and glycemia. Studies have shown that high serum ferritin is associated with an increased risk of T2DM independent of established diabetes risk factors and with iron levels below those found in hemochromatosis-associated diabetes [6,15]. In gestational diabetes, both BMI and serum ferritin levels were found to be independent predictors of 2 h glucose during an oral glucose tolerance test. Even in apparently healthy individuals, serum levels of ferritin were also positively correlated with oral glucose tolerance test. Studies have shown that poorly controlled diabetes, in both T1DM and T2DM, is associated with increased serum ferritin. In addition, ferritin has been shown to predict HbA1c independently of glucose in T2DM [16]. IR assessed either by the minimal model or euglycemic clamp was associated with total iron stores, even in the presence of normal glucose tolerance [16]. A recent cross-sectional survey in China that included 8235 patients showed that serum ferritin levels were associated with a higher risk of diabetes, higher levels of HbA1c, and HOMA-IR, independent of several confounders including age, sex, education, smoking, BMI, serum lipids, and hypertension [17]. An earlier survey in Germany conducted on a predominantly nondiabetic population showed a positive association between serum ferritin and elements of IR syndrome [18].

In the current study, the IR group differed significantly with regard to TG level, total cholesterol, BMI, waist circumference, and waist/hip ratio. These results go in harmony with the concept that considers the rising prevalence of IR in T1DM to reflect, in part, increasing rates of obesity among those patients [19].

A recent hypothesis (the accelerator hypothesis) claimed that excess adiposity and its consequences of glucotoxicity and accelerating β-cell apoptosis could lead to increased immunogenicity by triggering autoimmunity [20]. Interestingly, our results have revealed that C-peptide levels in the IR group, who had a significantly higher BMI and waist/hip ratio, were significantly lower than that in non-IR group. A

recent study demonstrated that with increasing BMI the proportion of children with preserved C-peptide was higher [21]. Difference between our study result regarding C-peptide and that of other studies could be related to the older age group included in this study. Some studies suggested that C-peptide decreases with prolonged disease duration, whereas others found residual function in old age with the use of ultrasensitive techniques [22,23].

Finally, in this study, hsCRP was higher in T1DM than in the healthy control group, and those with IR had a significantly higher level than non-IR patients. Several studies have investigated the utility of hsCRP for CV risk predication [24]. Although not endorsed as a CV risk predictive and prognostic marker by many associations [25], its relationship to CV risk has been demonstrated in several studies [24]. hsCRP was demonstrated in one study to be higher in T1DM patients with early signs of atherosclerosis [26]. In another study, hsCRP was also significantly elevated in T1DM diabetics compared with the healthy control group [27].

Emergence of IR among T1DM patients could increase the propensity for macrovascular complications. In this study, hepcidin had a neutral role among T1DM diabetic patients. Although comparative analysis and simple bivariate correlation have proven serum ferritin to be related to IR in these patients, the multivariate analysis has invalidated its role as a predictor of IR. Thereby, it is prudent to study ferritin further in T1DM patients with macrovascular complications, especially those with IR.

Study limitations

The sample size was relatively small, which was predetermined by our T1DM clinic regular attendee pool. In addition, smoking status was not recorded in our study, although the effect of smoking on ferritin levels increases with age [28].

Conclusion

Hepcidin plays no role in T1DM IR patients, yet ferritin levels were higher in this subset of patients and were negatively correlated with eGDR. However, multivariate regression analysis annulled this relation.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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