Association of serum ferritin with insulin resistance in offsprings of type 2 diabetics
Usama A. Khalil, Fayroz O. Seliem, Alsayed Alnahal, Mohamed Awad, Ayman M.E.M. Sadek, Mohamed S. Fawzy

Context
Type 2 diabetes is prevalent worldwide, and insulin resistance (IR) is considered the main player in its pathogenesis. Previous studies suggested a link between iron and IR.

Aim
The aim was to study serum ferritin level in nondiabetic offsprings, with and without impaired glucose tolerance, of diabetic patients and its relation to IR.

Settings and design
This is a cross-sectional case–control study carried out in the Internal Medicine Department, Zagazig University Hospitals.

Patients and methods
A total of 25 completely healthy individuals as a control group and 50 offsprings of patients with type 2 diabetes as a case group were included in the study. The case group was further divided into normal and impaired glucose tolerant offspring subgroups after glucose tolerance test. All of them underwent thorough clinical examination; routine laboratory investigation including complete blood count, liver and kidney function tests, fasting and postprandial blood glucose, serum ferritin, and fasting insulin by enzyme-linked immunosorbent assay; calculation of BMI; and homeostasis model assessment-estimated insulin resistance (HOMA-IR).

Statistical package for the social sciences for windows (version 16) was used for statistical analysis.

Results
Significant increase in mean±SD of serum ferritin, fasting insulin, HOMA-IR, and fasting and postprandial blood glucose levels in impaired glucose tolerant offspring subgroup was observed as compared with both control group and normal glucose tolerant offspring subgroup. Significant positive correlation was found between serum ferritin versus each of BMI, fasting insulin, fasting, postprandial blood glucose, and HOMA-IR in impaired glucose tolerant offspring subgroup.

Conclusion
Elevated serum ferritin levels in nondiabetic offsprings with impaired glucose tolerance may play a role in the pathogenesis of IR state, which may progress to type 2 diabetes.

Keywords:
ferritin, insulin resistance, type 2 diabetes

Introduction
The frequency of diabetes mellitus (DM) type 2 is expanding in each nation, with 80% of those with DM living in low-income and middle-income countries. DM caused 4.6 million deaths in 2011. It is suspected that by the year 2030 ~439 million individuals will have DM type 2 [1].

Insulin resistance (IR) occurs before leading to DM and might be the best indicator for it [2].

Past observational studies have proposed the potential role of iron in the pathogenesis of diabetes. Sensitivity for insulin and its secretion is expanded by frequent blood donation [3].

The molecular mechanisms are various and incompletely understood; however, it incorporates oxidative stress, modulation of adipokines, and intracellular singling transduction pathways [4]. The control of this vital yet conceivably lethal substance is crucial to human well-being and sickness [5].

Many tissues have ferritin as a cytosolic protein, yet little amounts of it are found in the serum where it transports iron. Clinically measureable ferritin in

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plasma is usually apoferritin, a noniron containing molecule that gives an indirect estimation of total iron storage of the body [6].

Studies have revealed a positive correlation between serum ferritin in individuals at risk of diabetes and patients with DM type 2 [7]. Another study had reported relative iron overload in offspring of type 2 diabetics as a link to IR [8].

The current work is aimed at studying the serum ferritin level in nondiabetic offsprings of patients with DM type 2, whether they had impaired glucose tolerance or not, and its relation to IR.

Patients and methods
This case–control study was carried out in the Internal Medicine and Medical Biochemistry Departments, Faculty of Medicine, Zagazig University, in the period from April 2015 to February 2016 and included 75 volunteers, who were allocated into two main groups.

Group A (control group) includes 25 apparently healthy male participants. Their ages ranged from 18 to 35 years, with mean values±SD of 24.28±4.57 years, and their BMI ranged from 17 to 24 kg/m², with mean values±SD of 21.64±1.7. They were nondiabetic (their fasting blood glucose level ranged from 75 to 96 mg/dl, with mean values±SD of 87.64 ±5.87 mg/dl, and their 2-h postprandial blood glucose level ranged from 110 to 139 mg/dl, with mean values ±SD of 121.24±8.5 mg/dl). They had no family history of DM, hypertension, or obesity.

Group B included 50 nondiabetic male offsprings whose one or both parents had type 2 DM and were on insulin or oral hypoglycemic drugs, and they were further subdivided into two subgroups according to oral glucose tolerance test. Group B1 included 25 male nondiabetic normal glucose tolerant offsprings of type 2 diabetic patients. Their ages ranged from 18 to 45 years, with mean values±SD of 25.68±6.52 years. Their BMI ranged from 20 to 24, with mean values±SD of 22.24±1.09. They had normal glucose tolerance (their fasting blood glucose level ranged from 70 to 95 mg/dl, with mean values±SD of 82.44±5.40 mg/dl, and their 2-h postprandial blood glucose level ranged from 110 to 139 mg/dl, with mean values±SD of 123.16 ±8.72 mg/dl). Group B2 included 25 healthy male nondiabetic impaired glucose tolerant offsprings of type 2 diabetic patients. Their ages ranged from 19 to 38 years, with mean values±SD 28.04±5.22 years, and their BMI ranged from 26 to 30, with mean values±SD of 27±1.22. They had impaired glucose tolerance (their fasting blood glucose level ranged from 107 to 124 mg/dl, with mean values±SD of 117.2±5.52 mg/dl, and their 2-h postprandial blood glucose level ranged from 140 to 195 mg/dl, with mean values±SD of 163.48±15.55 mg/dl).

Inclusion criteria
The inclusion criteria were as follows: age older than 18 years to younger than 65 years; male offspring, with normal or impaired glucose tolerance, of one or both parents who had type 2 DM and were on insulin or oral hypoglycemic drugs; and male participants with normal glucose tolerance and negative family history of diabetes as control group.

Exclusion criteria
The following exclusion criteria were applied: conditions that lead to elevated serum ferritin level like uncontrolled hypertension, liver and/or kidney diseases, thyroid disease, cardiovascular disease, any active inflammatory diseases, elderly age, and overt diabetes with fasting blood glucose more than 126 mg/dl and/or 2 h postprandial glucose level more than 200 mg/dl. Female sex was excluded owing to marked variability in serum ferritin levels according to menstruation status, pregnancy, lactation, and contraceptive medications intake, which makes data interpretation more confounded as compared with the male sex and makes comparisons unfair.

Ethical clearance
Written informed consent was taken from the participants to participate in the study. Approval for performing the study was obtained from Internal Medicine and Medical Biochemistry Departments, Zagazig University Hospitals, after taking Institutional Review Board (IRB) approval.

All participants of the study were subjected to full history and thorough clinical examination with special stress on family history of DM, hypertension, and obesity and calculation of BMI [BMI=weight (kg)/height (m²); classification of obesity was based on BMI] [9].

Routine investigations
It included urine analysis (for glucose, acetone, protein, pH, bilirubin, and leukocytes), complete blood picture, liver chemistry (serum bilirubin total and direct, serum albumin, serum alanine transferase, and serum aspartate transferase), renal function tests (serum
creatinine level and urea), sodium and potassium electrolyte levels, C-reactive protein by agglutination procedure, and oral glucose tolerance test [10].

Specific investigations
It included serum ferritin by high-sensitivity enzyme-linked immunosorbent assay [11], fasting insulin by enzyme-linked immunosorbent assay technique [12], and calculation of IR by homeostasis model assessment-estimated insulin resistance (HOMA-IR) index [HOMA index = fasting insulin (IU/ml)×fasting plasma glucose (mmol/l)/22.5] [13]. Participants were categorized as IR if their HOMA-IR was greater than 1.64 [14].

Sampling
After an overnight fasting (12 h), venous blood (8 ml) was collected from all participants under complete aseptic conditions and divided into three portions: 4 ml of blood collected on sodium fluoride–oxalate for centrifugation and plasma separation for fasting plasma glucose and routine laboratory examination. The remaining 4 ml of blood was collected in a plain tube and was left for 30 min, and then centrifuged for 10 min to separate the serum; this was subdivided into two plain tubes and kept in deep freezer at −70°C for determination of fasting insulin and serum ferritin. Two hours after meal, another blood sample was taken on fluoride–oxalate for measurement of 2-h postprandial blood glucose.

Statistical analysis
All data were coded, checked, entered, and analyzed using the standard version of SPSS for windows (version 16) of SPSS incorporation (SPSS Inc., Chicago, Illinois, USA) [15].

Results
Tables 1 and 2 show statistically highly significant increase in BMI (kg/m²), fasting blood glucose (mg/dl), 2-h postprandial blood glucose (mg/dl), fasting insulin (IU/ml), HOMA-IR, and serum ferritin (μg/l) among group B2 in comparison with group A and group B1; moreover, a statistically significant increase in insulin (IU/ml), HOMA-IR, serum ferritin (μg/l) in group B1 was observed as compared with group A. There was no statistically significant difference present in white blood cells, hemoglobin %, platelets, alanine transaminase, aspartate transaminase, creatinine, and albumin among the studied groups. Table 3 shows a statistically significant positive correlation between serum ferritin and BMI (kg/m²), fasting blood glucose, 2-h postprandial blood glucose, insulin, and HOMA-IR in group B2, whereas no statistically significance was detected in group A and group B1.

Discussion
International Diabetic Federation estimates 34.6 million people with diabetes in Middle East and

### Table 1 Comparison of mean value±SD of age, clinical, and routine laboratory parameters among the studied groups and subgroups of the study

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<tbody>
<tr>
<td>Age (years)</td>
<td>24.28±4.57</td>
<td>25.68±6.52</td>
<td>28.04±5.22</td>
<td>2.98</td>
<td>0.056</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.64±1.7</td>
<td>22.24±1.09</td>
<td>27.00±1.22</td>
<td>115.58</td>
<td>&lt;0.001</td>
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<td>Albumin (g/dl)</td>
<td>3.7±0.52</td>
<td>3.6±0.61</td>
<td>3.8±0.52</td>
<td>0.84</td>
<td>0.44</td>
</tr>
<tr>
<td>ALT (μl/l)</td>
<td>33.2±7.64</td>
<td>34.3±10.41</td>
<td>37±7.63</td>
<td>1.44</td>
<td>0.24</td>
</tr>
<tr>
<td>AST (μl/l)</td>
<td>18.4±1.89</td>
<td>17.4±2.29</td>
<td>18.1±2.5</td>
<td>1.26</td>
<td>0.29</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.06±0.19</td>
<td>0.98±0.28</td>
<td>0.90±0.27</td>
<td>2.65</td>
<td>0.09</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12±0.91</td>
<td>12.3±0.52</td>
<td>12.5±0.94</td>
<td>1.16</td>
<td>0.32</td>
</tr>
<tr>
<td>WBCs (×10³/μl)</td>
<td>5.2±1.19</td>
<td>5.8±1.36</td>
<td>5.4±1.58</td>
<td>1.25</td>
<td>0.29</td>
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ALT, alanine transaminase; AST, aspartate transaminase; Hb, hemoglobin; WBC, white blood cells. aSignificant regarding group A. bSignificant regarding group B1. cAnalysis of variance test.

### Table 2 Comparison of mean value±SD of specific investigation among the studied groups and subgroups of the study

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<tr>
<td>CRP (mg/l)</td>
<td>3.14±1.75</td>
<td>2.42±1.48</td>
<td>2.12±0.93</td>
<td>3.05</td>
<td>0.06</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>82.44±5.40</td>
<td>87.64±5.87</td>
<td>117.2±5.52</td>
<td>297.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2-h postprandial blood glucose (mg/dl)</td>
<td>121.24±8.5</td>
<td>123.16±8.72</td>
<td>163.48±15.55</td>
<td>109.39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin (IU/ml)</td>
<td>2.96±1.37</td>
<td>7.08±1.44</td>
<td>8.24±1.09</td>
<td>112.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.62±0.27</td>
<td>1.39±0.29</td>
<td>2.36±0.25</td>
<td>262.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum ferritin (μg/l)</td>
<td>207.36±48.4</td>
<td>369.12±48.16</td>
<td>692.96±125.96</td>
<td>223.37</td>
<td>&lt;0.001</td>
</tr>
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CRP, C-reactive protein; FBG, fasting blood glucose; HOMA-IR, homeostasis model assessment-estimated insulin resistance. aSignificant as regards to group A. bSignificant as regards to group B1. cAnalysis of variance test.
North Africa, with a prevalence rate of 10.9%. In Egypt, 42% of diabetic patients experience early stages of eye disease and 5% are legally blind [16].

We aimed in this study to explore linkage between level of serum ferritin and IR on one hand and the risk of hyperferritinemia on developing diabetes in nondiabetic offsprings of patients with DM type 2 on the other hand.

It revealed that overweight offsprings of those with DM type 2 had a significant high serum ferritin, which was positively correlated with BMI in impaired glucose tolerance participants, supporting the results of Wrede et al. [17] and Zafar et al. [18], who reported significant increase in serum ferritin values with high BMI (>25 kg/m²). Moreover, Jaganatha et al. [19] found its significant positive correlation with BMI. However, Pramiladevi et al. [20] reported the absence of such correlation, and the difference from our study might be explained by the different age and sex distribution, as the present study had excluded female gender to avoid the wide variation that occurs in ferritin level according to hormonal status, supporting what Sazandeh reported [7].

The current study showed significant increase in each of serum ferritin, fasting blood glucose, 2-h postprandial blood glucose, and HOMA-IR in impaired glucose tolerance nondiabetic offspring as compared with the controls and normal tolerant offspring, with no significant difference in C-reactive protein levels between the three groups of study. This goes in agreement with the results obtained by Sharifi and Sazandeh [7], who reported that impaired glucose tolerance participants had a significant increased ferritin compared with control group, implying that hyperferritinemia occurs before elevation of plasma glucose concentration. Moreover, Smotra and Kudyar [21] reported that increased serum ferritin levels, which reflect body iron stores, had a significant positive correlation with serum insulin levels.

Koo et al. [22] found that the possible increased risk of DM by the effect of hyperferritinemia might be through increased IR rather than dysfunctional beta cells. In addition, Facchini [23] also found a significant decrease in serum insulin concentration and improvement in insulin sensitivity after performing phlebotomy, and also Canturk et al. [24] confirmed hyperferritinemia in poorly controlled diabetics. Moreover, Fernandez-Real et al. [25] concluded the possible association of glucose intolerance, type 2 diabetes, and gestational diabetes with increased body iron stores.

Jaganatha et al. [19] found a significantly higher serum ferritin level with higher fasting insulin level, with a positive correlation with the duration of diabetes. Increased serum ferritin is strongly associated with current or future diabetes development in individuals at risk of diabetes. Pramiladevi et al. [20] found that there was a significant correlation in diabetics compared with individuals with normal blood sugar regarding increased serum ferritin, and hyperferritinemia may be one of the causes for development of IR before overt diabetes. On the contrary, Zafar et al. [18] found no association between ferritin levels and IR markers in diabetic patients. However, a significant positive correlation between serum transferrin saturation and IR was observed. This can be explained by the difference in age, sex, sample size, environmental, and nutritional status from the population in the present study.A significant positive correlation was found in the current study between serum ferritin level and each of fasting blood glucose, IR, 2-h postprandial blood glucose, and HOMA-IR in the impaired glucose tolerance offsprings but not in the control and normal glucose tolerance offsprings, which suggests the role of serum ferritin elevation on IR, supporting the results of Sazandeh [7] and Wrede et al. [17] who reported the significant correlation of IR criteria with serum ferritin in a large representative population. Moreover, Suvarna et al. [26] stated similar indirect evidence that IR correlated well with the total units of

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<td></td>
<td>R</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.14</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>0.39</td>
<td>0.06</td>
<td>0.08</td>
</tr>
<tr>
<td>2 h postprandial (mg/dl)</td>
<td>0.17</td>
<td>0.43</td>
<td>0.34</td>
</tr>
<tr>
<td>Insulin (IU/ml)</td>
<td>-0.21</td>
<td>0.32</td>
<td>0.39</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>-0.08</td>
<td>0.69</td>
<td>0.38</td>
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FBG, fasting blood glucose; HOMA-IR, homeostasis model assessment-estimated insulin resistance.
blood taken in chronically transfused patients with thalassemia major. Desferal improved IR in patients with uremia with iron overload [27].

We can conclude that elevation of serum ferritin in nondiabetic offsprings of patients with type 2 diabetes may play a role in IR with subsequent susceptibility to type 2 DM. Further studies are needed to verify the importance of screening of hyperferritinemia in offsprings of type 2 diabetic patients and to define cutoff level of serum ferritin for possible early detection and subsequent prevention or delaying of impaired glucose tolerance and diabetes in those participants.

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Conflicts of interest
There are no conflicts of interest.

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