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Research Article

Effect of iron on pancreatic beta cell function and insulin resistance in female albino rats

E.O. Adewoye*, O.I. Adeyomoye and A.O. Ige
Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria

Keywords:
Iron, Hyperglycaemia, Insulin resistance, Hyperinsulinaemia, inflammation, Beta cell dysfunction.

ABSTRACT

Background: Increase in total body iron store has been reported in the aetiology and development of diabetes mellitus. The effect of iron supplementation in female with respect to the incidence of diabetes mellitus was investigated on the pancreatic beta cell function and insulin resistance in normal female rats. **Methods**: Forty-eight Wistar rats (150-200g) were divided into 6 groups as follows; Group 1 (control) received 0.3ml distilled water, groups 2, 3, 4, 5 and 6 received iron (10mg/kg, 20mg/kg, 40mg/kg, 80mg/kg and 160mg/kg respectively) daily for 12days. Blood from the tail vein of each animal was assessed for blood glucose level on days 0, 3, 6 and 12 using glucometer. At 12 days post iron treatment, blood (3ml) was obtained from the retro-orbital sinus of each animal and allowed to coagulate. Serum obtained was analysed for insulin concentration using ELISA method. Histopathology of the pancreas was assessed using Hematoxylin and Eosin technique. Data were expressed as Mean ± SEM and analyzed using two-way ANOVA at $P<0.05$. **Results**: Blood glucose level, insulin concentration, insulin resistance and pancreatic beta cell function increased significantly with increased concentration of iron. Histology of the pancreas showed fat infiltration of both acini and islets with increased iron concentration. Mild inflammation of the islets was observed at 160mg/kg. **Conclusion**: Administration of iron at 40mg/kg and above in female rats caused hyperglycaemia, insulin resistance, hyperinsulinaemia, inflammation and pancreatic beta cell dysfunction thus predisposing the animal to type 2 diabetes mellitus.

INTRODUCTION

Iron is a vital metal required for life. It has the ability to donate and to accept electrons therefore the regulation of this necessary but potentially toxic substance is important in health and disease. Generation of reactive oxygen species (ROS) by metal oxidants such as iron have been reported to be involved in cell damage (De Zwart *et al.*, 1999), steatohepatitis and Alzheimer's disease (Chritturis *et al.*, 2001; Barbagallo *et al*., 2013). Iron overload has been reported to contribute to increased free radical formation while increased oxidative stress has been implicated in hemochromatosis and alpha thalasemia (Livrea *et al.*, 1996). However, iron deficiency is a common disorder seen in clinical medicine and severe cases may result in anaemia and impairment of physical performance despite the adaptive changes within the red blood cells which facilitate oxygen delivery (Kotze *et al*., 2009).

The widespread concern with the morbidity of severe iron deficiency is reflected in prophylactic programmes of iron supplementation and fortification (Verdon *et al.*, 2003). The use of iron supplementation is common among females who often lose iron through normal reproductive activities (Petterson *et al.*, 2001, Verdon *et al.*, 2003, Vaucher *et al.*, 2012) and this is often abused. In diabetes mellitus, it has been reported that iron depletion therapy may be beneficial for improving the efficiency of glycemic control (Adel *et al*., 2015). Qiu *et al*, (2011) also reported that menstruation could exert a protective effect in diabetes mellitus by preventing iron overload in premenopausal women. Though excess iron could be a risk factor in the development of type 2 diabetes mellitus, its deficiency could also be deleterious. It is therefore important to have a balance between need and overload to minimize

Address for correspondence:
E-mail: elolade@yahoo.com
Tel.: +2348023405879

the risk of damage to tissues. The mechanisms by which excess iron constitute a risk factor in the development of diabetes mellitus have not been fully elucidated; this study was therefore designed to assess the effect of iron supplementation at different concentrations on pancreatic beta cell function and insulin resistance in female wistar rats.

MATERIALS AND METHODS

Animals

Forty-eight albino Wistar rats (150-200g) were obtained from the Central Animal House, College of Medicine, University of Ibadan. They were housed in well aerated cages, fed on standard rat chow and allowed free access to drinking water according to the guidelines and regulations of National Institute of Health (NIH) (NIH Publication, 85-23, 1985). The rats were randomly divided into six groups of eight rats each as follows: group 1 (Control) received 0.3ml distilled water; groups 2-6 were treated with 10mg/kg, 20mg/kg, 40mg/kg, 80mg/kg, and 160mg/kg ferrous sulphate (Manufactured by Nostrum Pharmaceutical Limited, Lagos) respectively for 12days. All treatments were given orally using orogastric tube.

Blood Sample and Analysis

Blood samples were obtained by making small cuts on the tip of tail on days 0 (pre-treatment), 3, 6 and 12 and analysed for fasting blood glucose level using the Fine Test Glucometer. On the 12th day post-iron treatments, blood samples were also obtained from the retro-orbital sinus (after light anaesthesia with di-ethyl ether) into clean tubes using non-heparinized capillary tubes. Serum obtained from these blood samples were centrifuged at 3000g for 10mins and analysed for insulin concentration using Enzyme Linked Immunosorbent Assay (ELISA) method as described by Yalow *et al.*, (1960).

The Homeostasis Model Assessment Insulin Resistance (HOMA-IR) and Pancreatic Beta Cell Function (HOMA-β) were determined mathematically using the following equations

$$\text{HOMA-IR} = \frac{\text{Insulin (µU/ml)} \times \text{Glucose (mg/dl)}}{405}$$

$$\text{HOMA-}\beta = \frac{360 \times \text{Insulin (µU/ml)}}{\text{Glucose (mg/dl)} - 63}$$

(Mathew *et al.*, 1985)

Histopathology of the Pancreas

The pancreas of each animal was harvested after cervical dislocation and fixed in 10% formalin for 48hrs. The tissues were embedded in paraffin wax, sectioned to a thickness of 4µm and stained using the Haematoxylin and Eosin technique (Steven *et al*, 2001).

Statistical Analysis

Results obtained are expressed as Mean ±SEM and the level of statistical significance was taken at $p<0.05$ using the two-way analysis of variance (ANOVA).

RESULTS

Effect of Iron on Blood Glucose Concentration

There was significant increase ($p<0.05$) in blood glucose concentration in 40mg/kg and 160mg/kg iron treated groups when compared to control on day 3. There was significant increase in blood glucose level in 80mg/kg and 160mg/kg iron treated groups when compared to control on day 6 (Table 1). By day 12 of iron administration, all treated groups showed significant increase in blood glucose level when compared to day 0 (Pre-treatment). At day 12 post-treatment, percentage increases in blood glucose level in the 10mg/kg, 20mg/kg, 40mg/kg, 80mg/kg and 160mg/kg iron treated, were 20.8%, 19.5%, 22.9%, 21.5%, and 25.86% respectively when compared with the control group.

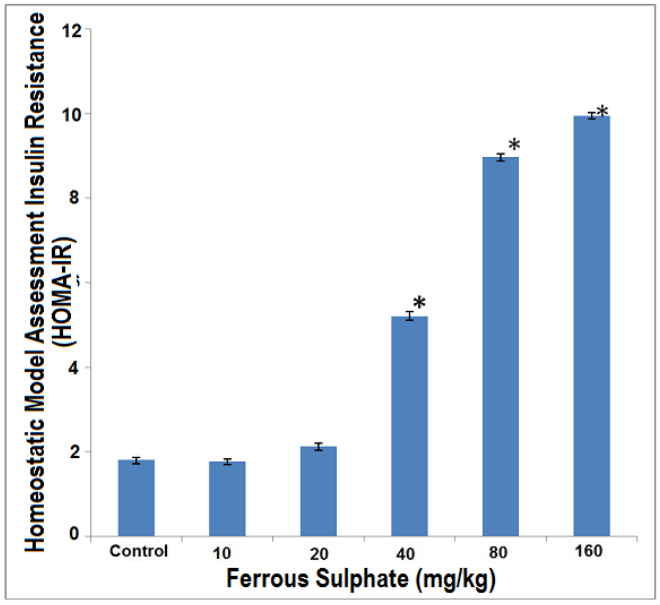

Fig 1: Homeostasis Model Assessment Insulin Resistance (HOMA-IR) in Control and Ferrous Sulphate treated rats. * indicates values that are significantly different from control. $p<0.05$ n=8

Effect of Iron on Insulin Concentration in Control and Treated Rats

The results showed that animals treated with 10mg/kg and 20mg/kg iron showed no significant difference in insulin concentration when compared with the control while significant differences ($p<0.05$) occured in groups treated with 40mg/kg, 80mg/kg and 160mg/kg, with percentage Insulin increase of 63.7%, 69.8% and 78.95% respectively when compared with control (Table 2).

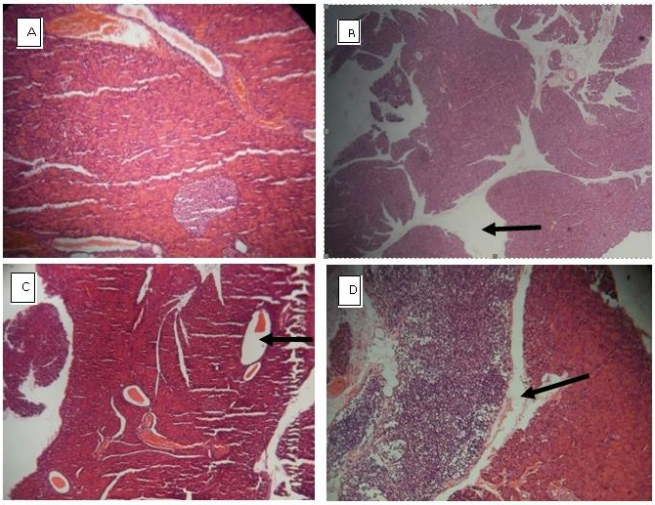

Plate 1: Photomicrograph of the Pancreas in Control and Ferrous Sulphate Treated Animals (x40): A (Control) shows the normal architecture of the pancreas while B, C and D (40mg/kg, 80mg/kg,160mg/kg ferrous sulphate treatment groups) show pancreas infiltrated with fat deposits (Black Arrows)

Effect of Iron on Insulin Resistance in Control and Treated Rats

The changes observed in animals treated with 10mg/kg iron were not statistically significant when compared with control group. There was significant increase of 12.2%, 63.49%, 79.12% and 80.83% in insulin resistance in animals treated with 20mg/kg 40mg/kg, 80mg/kg and 160mg/kg respectively when compared with the control (Fig 1).

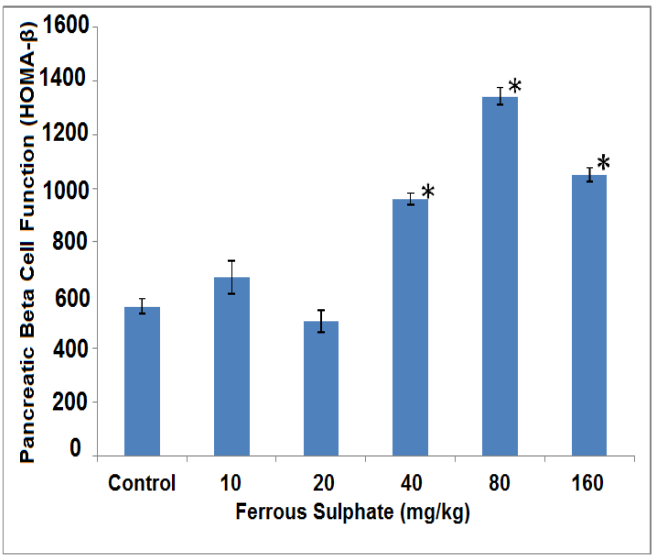

Fig 2: Pancreatic beta cell function (HOMA-β) in Control and Ferrous Sulphate treated rats. *indicates values that are significantly different ($p<0.05$) from control group. n=8

Effect of Iron on Beta Cell Function in Control and Treated Rats

The results show no significant difference in pancreatic beta cell function in animals treated with 10mg/kg and 20mg/kg when compared with control group. Animals treated with 40mg/kg, 80mg/kg and 160mg/kg iron show significant increase ($p<0.05$) in pancreatic beta cell function when compared with control with a percentage increase of 16.7%, 54.9% and 60.26% respectively (Fig 2).

Histological Assessment of the Pancreas in Control and Treated Groups.

Plate 1A: shows the normal architecture of the pancreas in the control group. Plate 1B, 1C and 1D show fat deposition on the pancreas (Black Arrows) at 40mg/kg, 80mg/kg and 160mg/kg respectively

Plate 2A: shows the normal architecture of the islet cells in the control group. Plate 2B, 2C and 2D show infiltration of the islet cells by fat deposits (Black Arrows) at 40mg/kg, 80mg/kg and 160mg/kg respectively. Treatments with iron at 40mg/kg, 80mg/kg and 160mg/kg caused an infiltration of the islet cells by fat deposits (Black Arrows). In animals receiving 160mg/kg iron, a mild inflammation of the cells was also observed in the islet cells (White Arrow) (Plate 2D).

Plate 3A shows normal architecture of the exocrine acini in the control group. Treatments with iron at 40mg/kg, 80mg/kg and 160mg/kg caused distortion of the exocrine acini and fat infiltration (Black Arrows) Plate 3B, 3C and 3D.

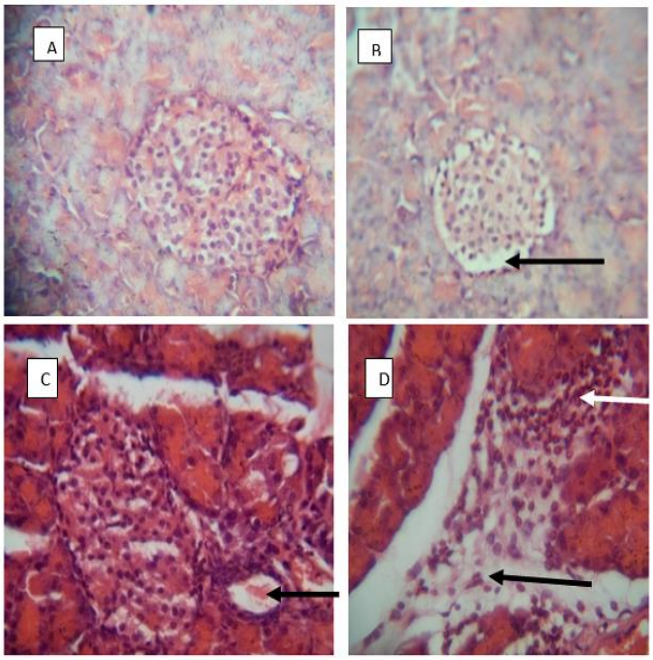

Plate 2: Photomicrograph of Islet Cells in Control and Ferrous Sulphate Treated Animals (x400): A (Control) shows the normal architecture of the islet cell, while B, C and D (40mg/kg, 80mg/kg and 160mg/kg ferrous sulphate treatment groups) show the islet cells infiltrated with fat deposit (Black Arrows). D (160mg/kg ferrous sulphate treatment group) shows mild inflammation of the islet cells (White Arrow)

Table 1: Blood Glucose Concentration in Control and Ferrous Sulphate treated rats

Treatment Groups	Blood glucose level(mg/dl) Day 0 (pre-treatment)	Blood glucose level (mg/dl) Day 3 (Treated)	Blood glucose level(mg/dl) Day 6 (Treated)	Blood glucose level (mg/dl) Day12 (Treated)
Control group	60.00 ± 3.33	66.00 ± 3.51	65.00 ± 3.03	62.00 ± 1.62
10mg/kg/day Ferrous sulphate treated	64.25 ± 2.81	77.75 ± 3.22	77.00 ± 2.76	78.25 ± 2.56*
20mg/kg/day Ferrous sulphate treated	63.75 ± 2.16	76.38 ± 4.08	76.88 ± 4.39	77.00 ± 3.33*
40mg/kg/day Ferrous sulphate treated	62.75 ± 2.80	83.50 ± 5.80*	75.75 ± 2.72	80.38 ± 5.80*
80mg/kg/day Ferrous sulphate treated	66.75 ± 3.17	80.63 ± 4.19	82.13 ± 4.30*	79.00 ± 3.85*
160mg/kg/day Ferrous sulphate treated	66.13 ± 2.57	84.88 ± 3.50*	90.25 ± 5.07*	83.63 ± 2.48*

Values are expressed as Mean ± SEM. * Indicates values significantly different from control. $p < 0.05$ n=8

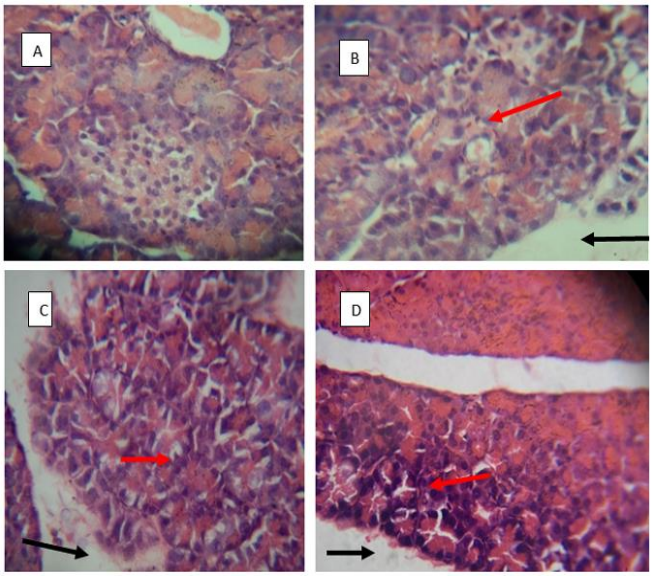

Plate 3: Photomicrograph of the Exocrine Acini in Control and Ferrous Sulphate treated rats (x400): A (Control) show the normal architecture of the exocrine acinar while B, C and D (40mg/kg, 80mg/kg and 160mg/kg ferrous sulphate treatment groups) show distorted exocrine acini (Red Arrows) with fat infiltration (Black Arrows)

DISCUSSION

Diabetes mellitus is a metabolic disorder characterized by disturbances in carbohydrate, protein and lipid metabolism (American Diabetes Association 2012). A persistent increase in fasting blood glucose level above normal may lead to the development of diabetes mellitus (Ahmed *et al*., 2015). In this study, oral iron supplementation at all doses used caused an increase in fasting blood glucose level. This result is consistent with the report of Tommi-pekka *et al*. (2010) who had earlier reported the hyperglycaemic effect of iron. It is therefore possible that the oral iron supplementation at the doses used might have caused an increase in glucose production.

Table 2: Fasting Insulin Concentration in Control and Ferrous Sulphate treated rats

Groups	Insulin Concentration (µU/ml)
Group 1 Control (0.3ml distilled water)	9.50±0.84
Group 2 (10mg/kg/day Ferrous sulphate treated)	10.23±1.79
Group 3(20mg/kg/day Ferrous sulphate treated)	10.86±1.34
Group 4 (40mg/kg/day Ferrous sulphate treated)	26.19±3.11*
Group 5 (80mg/kg/day Ferrous sulphate treated)	31.48±4.90*
Group 6 (160mg/kg/day Ferrous sulphate treated)	45.15±2.59*

***** indicates values that are significantly different from control. $p<0.05$ n=8

Insulin is a hormone which suppresses the elevation of plasma glucose level by promoting glucose uptake into cells and inhibiting glucose release from the liver (Longnecker *et al*., 2014). The peripheral tissues of the body develop resistance when there is decrease

sensitivity to insulin (Kahn *et al*., 2006). Therefore, absence or deficient action of insulin in target tissues may result in hyperglycaemia and abnormalities in carbohydrates, protein and lipid metabolism (Daniel *et al.*, 2013). Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) is generally considered as an index used to assess insulin resistance while the Homeostasis Model Assessment-β (HOMA-β) is used to assess pancreatic beta cell function in animal models (Mathews *et al.,* 1985; Weir *et al*., 2001). Using the HOMA-IR index, hyperinsulinaemia and increased insulin resistance was observed in the 40mg/kg, 80mg/kg and 160mg/kg iron treated animals respectively which suggests an alteration of glucose transport in adipocytes, liver and muscle cells as earlier reported by Green *et al. (*2006). In addition to this, an analysis of Beta function using the HOMA-β index indicates an increase in pancreatic beta cell function of the 40mg/kg, 80mg/kg and 160mg/kg iron treated groups. It has been suggested that iron accumulation contributes to increased free radical formation and oxidative stress which can result in cell damage (Halliwell *et al* 1999; Chitturi *et al*., 2001). However, iron has also been reported to act as co-factor for primary anti-oxidant activities (Mainous *et al*., 2005). It is therefore likely that increased pancreatic beta cell function observed in this study at 40mg/kg, 80mg/kg and 160mg/kg could be due to the effect of iron in potentiating antioxidants defence mechanism by acting as a co-factor (Mainous *et al*., 2005). This implies that iron, though deleterious when it is in excess, could also have protective effect when moderated. Hyperglycaemia as observed in diabetes mellitus causes oxidative stress which has been reported to trigger the release of several inflammatory markers such as interleukin-6 (IL-6), C-reactive proteins and tumour necrosis factor-alpha (TNF-α) (de Rekeneire *et al*., 2006). These inflammatory markers have been reported to exert deleterious effects on various organs in the body such as pancreas, liver, brain, muscle and heart tissue (Horng *et al*., 2013). In the pancreas, the inflammatory process is characterised by the presence of cytokines, apoptotic cells, immune cell infiltration, amyloid deposits, fat deposits and eventually fibrosis (Donath *et al*., 2008; Nugent *et al*., 2008). The infiltration of islet cells by fat deposit in the 40mg/kg, 80mg/kg and 160mg/kg treatment groups in this study is characteristic of end-stage diabetes mellitus as reported by Nugent *et al*. (2008). Further to this, at 160mg/kg iron treatment, inflammation was observed suggesting a potentiation of inflammatory activities within the pancreas as iron concentration increased. This may therefore account for the reduction in pancreatic beta cell function observed in this treatment group (Fig 2). Iron overload therefore is a significant risk factor for metabolic syndromes (Gillum *et al*., 2001) and type 2 diabetes mellitus (Afkhami-Ardekani *et al*., 2008)

CONCLUSION

In conclusion, oral administration of iron at 40mg/kg and above in female rats caused hyperglycaemia, insulin resistance, hyperinsulinaemia, inflammation and pancreatic beta cell dysfunction thus predisposing the animal to type 2 diabetes mellitus. Therefore, the use of iron supplements should be taken with caution as it can be a significant risk factor for development of type 2 diabetes mellitus.

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