ASSOCIATION BETWEEN BODY IRON STORES AND GLUCOSE HOMEOSTASIS IN THE KUOPIO ISCHEMIC HEART DISEASE STUDY DATA

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ABSTRACT

Iron is a transition metal capable of catalyzing redox reactions by generating free radicals. These free radicals generated damage cells by attacking cell membranes, proteins and deoxyribonucleic acids. This damage can occur in the beta cells of the pancreas leading to reduced insulin secretion and increased insulin resistance leading ultimately to abnormal glucose homeostasis and diabetes mellitus. The aim of this study was to determine if there is any association between body iron stores and glucose homeostasis.

This study is part of the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD), which is an ongoing population based cohort study designed to investigate the risk factors of cardiovascular disease and other chronic illnesses in the middle age and aging men and women in eastern Finland using the 11- year follow-up data.

A total of 1774 subjects were assessed for this study, after excluding patients based on exclusion criteria and those with missing variables, we analyzed data from 1555 subjects, the mean age was 62.7 ± 6.5 years, while the mean serum ferritin concentration was 97.1 µg/L.

The study showed that body iron stores was associated with glucose homeostasis as assessed by Homeostasis model assessment using insulin resistance (HOMA-IR) and Homeostasis model assessment using beta cell function (HOMA-BcF). There was an inverse relationship between blood donation and HOMA-IR (β = -0.049, P= 0.024), while there was also an inverse relationship though not statistically significant between blood donation ever and HOMA-BcF (β = -0.020, P = 0.4).
Additional analysis for subjects who had 2 more donations a year showed a positive association with BcF ($\beta = 0.046$, $P = 0.770$) and insulin resistance ($\beta = 0.280$, $P = 0.021$).

Encouraging blood donation among healthy adults could provide additional benefits by improving the indices of glucose homeostasis even before overt symptoms and signs of abnormal glucose balance becomes apparent.
ABBREVIATIONS

ATP-adenosine triphosphate
BMI-body mass index
CVD-cardiovascular disease
DMT-divalent metal transporter
DNA-deoxyribonucleic acid
ETC-electron transport chain
HDL-high density lipoprotein
HOMA BcF-homeostasis model assessment using beta cell function
HOMA-IR-homeostasis model assessment using insulin resistance
IGT-impaired glucose tolerance test
KIHD-Kuopio ischemic heart disease study
LDL-low density lipoprotein
SSPG-steady state plasma glucose
TIBC-total iron binding capacity
UIBC-unsaturated iron binding capacity
WHO-world health organization
ACKNOWLEDGEMENT

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1) INTRODUCTION

Iron is a redox active transition element capable of accepting and donating electrons and as such is an important component of many vital biochemical reactions in the human body such as the electron transport chain (ETC). Iron is also capable of generating free radicals by its ability to participate in the Fenton reaction. These radicals can damage cells by attacking cell membranes, deoxyribonucleic acids (DNA), and proteins (Andrews. 1999). This damage can occur in the beta cells of the pancreas which secrete insulin resulting in decreasing secretion of insulin with consequent insulin lack and resistance that can lead to abnormal glucose balance and diabetes mellitus.

Dietary sources of iron include both animal and plant sources, although animal sources in the form of heme iron are the richest sources of iron and best absorbed by the human body (THL, 2000). Iron deficiency is a fairly common condition, Looker et al reported that around 3 percent of toddlers and 2 to 5 percent of American teenage girls are sufficiently iron deficient to have anemia (Looker et al.1997).

Iron deficiency is more common especially in the developing countries that might lack heme sources of iron in diet, it is estimated that more than half a billion people worldwide have adverse effects as a result of iron deficiency (Andrews. 1999). Iron overload on the other hand is commoner in the western world with the genetic condition of hereditary hemochromatosis being the primary cause classical. These patients with hereditary hemochromatosis have a defective gene, mutation C282Y in the HFE gene that ultimately leads to unregulated absorption of iron from the duodenum with consequent deposition of iron in the liver, pancreas, heart, pituitary, parathyroid glands and skin. It is estimated that in the United States around 1 in 10 Caucasians carries at least one allele with this mutation (Edwards et al. 1988). In Finland, data suggest the prevalence of hemochromatosis is about 50/100,000 (Karlsson. 1988).

Abnormal glucose balance and diabetes mellitus are commonly observed disease conditions worldwide, and the trend has been on the increase with about 30 million affected in 1985 to 177 million people by 2000 (Fauci, et al. 2008). Type 1 diabetes mellitus is commonest in Scandinavia with Finland having a prevalence of 35/100,000 per year (Fauci et al .2008). In Finland the prevalence of type 2 diabetes mellitus has been on the increasing trend, among people aged 30 years and above the incidence was 16/1,000 in a survey conducted between
1966 - 1972, 29/1,000 in 1976 and 32/1,000 in 1980 (Antti Reunanen, 1983). The prevalence of abnormal glucose tolerance and diabetes mellitus in Finland is 5.7% in men and 4.6% in women while the prevalence of impaired glucose tolerance (IGT) was 3.1% in men and 5.1% in women (Tuomilehto, 1991). A study by Saaristo et al. (Saaristo et al 2008) found that the prevalence of abnormal glucose tolerance among middle aged individuals was high, 42% in men and 33% in women; this high prevalence suggests that undetected glucose imbalance remains high.

The role of iron in the pathophysiology of abnormal glucose balance and diabetes mellitus cannot be underscored as presented in a number of studies (Salonen et al. 1998, Jiang, 2004, Forouhi, 2007, Fumeron, 2006). Therefore if excess body iron is involved in the pathogenesis of abnormal glucose balance, it could mean manipulating body iron stores could lead to corresponding changes in glucose balance and maybe also in the prevalence of diabetes mellitus. If body iron stores can be reduced by blood donation, we might be able to observe better parameters of glucose balance.

Jose Manuel Fernandez-Real et al concluded in their study of 181 healthy Caucasian men that those who were regular blood donors defined as those with two or more blood donations in the last 5 years had statistically significant increases in insulin sensitivity and decreased insulin secretion compared to non-donors. This association also clearly correlated with reduced serum ferritin in the blood donor group (Fernandez-Real, 2005). Iron supplementation was found to be associated with significant increases in insulin resistance (HOMA-IR) among pregnant women (Bo S, 2009). Lower iron stores and lower insulin resistance were observed among vegetarians as compared to non-vegetarians and this is postulated to be attributed to increased iron intake among non-vegetarians (Hua, 2001). Jian et al also concluded from their study that heme iron intake from red meat was positively associated with increased risk of DM (Jiang, 2004).

We intend to study the above association using a cohort of healthy Finnish men and women from the KIHD study and assessing their body iron stores and glucose homeostasis parameters. The study will be carried out using the cohort from the Kuopio ischemic heart disease risk factor study (KIHD), which is an ongoing study among middle aged men in eastern Finland to study risk factors for ischemic heart disease and atherosclerosis. It may then be plausible to presume that if significant changes in glucose balance parameters are observed among blood donors as compared to non-donors, it might be beneficial to
recommend blood donation among other things as both a preventative and educational tool for patients at increased risk of abnormal glucose balance and diabetes mellitus.
2 LITERATURE REVIEW

2.1 DEFINITION

Iron is an important element in human physiology and is found to be involved in various chemical reactions in living organisms. The primary ability to do this is because iron can accept and also donate electrons switching between its reduced state Fe²⁺ and its oxidized state Fe³⁺. It is thus an important component of many enzymes, serving as co-factor and also an integral component of oxygen binding molecules such as myoglobin and hemoglobin (Conrad, 2000).

Iron through this same ability also has the potential of damaging tissues by generating free radicals via the Fenton reaction. (Andrews, 1999) Iron thus has to be tightly controlled in order to avoid this damage. Iron is primarily bound to plasma transferrin as a transport medium in plasma and is stored in tissues as ferritin or hemosiderin. (Andrews, 1999). Disorders in this control results in either iron deficiency leading to anemia or iron overload as typified by hemochromatosis.

Iron is stored in muscle as myoglobin, circulating red blood cells as hemoglobin and also in the liver, pancreas, heart muscle and skin. It participates in hematopoiesis and is physiologically lost via desquamation of mucosal surfaces of the gastro-intestinal system and menstrual loss in pre-menopausal women. (Andrews, 1999).

2.2 SOURCES OF IRON

Iron is not homogenously produced by the human body and as such has to be derived. Iron is usually derived from dietary sources usually animal sources but also plant sources. Animal sources of iron contain heme iron which is better absorbed while plant sources contain non-heme iron which is not as efficiently absorbed as heme iron. It is usually recommended to consume non-heme iron with vitamin C or sources of Vitamin C to increase its absorption (Miret et al. 2003).
Table 1 below showing common sources of dietary iron in typical Finnish diet.

<table>
<thead>
<tr>
<th>Food types</th>
<th>Content/ portion (mg)</th>
<th>Portion mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pork liver</td>
<td>31.4</td>
<td>100</td>
</tr>
<tr>
<td>Semper breakfast cereal with rice and corn wheat</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>Nesquik breakfast cereal with cocoa</td>
<td>11.9</td>
<td>100</td>
</tr>
<tr>
<td>Kellogg’s special k classic breakfast cereal with rice wheat</td>
<td>11.6</td>
<td>100</td>
</tr>
<tr>
<td>Casserole with liver and rice</td>
<td>4.2</td>
<td>100</td>
</tr>
<tr>
<td>Finnish Easter pudding</td>
<td>1.9</td>
<td>100</td>
</tr>
<tr>
<td>Roasted barley porridge with water</td>
<td>1.4</td>
<td>100</td>
</tr>
<tr>
<td>Potato minced meat casserole, beef steak</td>
<td>1.3</td>
<td>100</td>
</tr>
<tr>
<td>Oat flake porridge with apple and cinnamon</td>
<td>1</td>
<td>100</td>
</tr>
</tbody>
</table>

Modified from the National Institute of Health and Welfare, Nutrition Unit.2013

Iron is also increasingly provided through supplements as iron sulphate, fumarate or gluconate. Iron supplements are also prescribed to pregnant women in the second and third trimester because of increased body iron requirements.
### Table 2: Recommended daily dietary allowance of iron is shown in the table below

#### Recommended dietary guidelines for iron in the US

<table>
<thead>
<tr>
<th>Category</th>
<th>Age Group</th>
<th>Recommended Intake (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>Adult</td>
<td>8</td>
</tr>
<tr>
<td>Women</td>
<td>Adult (age 50 and older)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Adult (ages 19 to 50)</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Pregnant</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Lactating</td>
<td>9 mg to 10 mg</td>
</tr>
<tr>
<td>Adolescents (ages 9 to 18)</td>
<td>Girls</td>
<td>8 mg to 15 mg</td>
</tr>
<tr>
<td></td>
<td>Boys</td>
<td>8 mg to 11 mg</td>
</tr>
<tr>
<td>Children (birth to age 8)</td>
<td>Ages 4 to 8</td>
<td>10 mg</td>
</tr>
<tr>
<td></td>
<td>Ages 1 to 3</td>
<td>7 mg</td>
</tr>
<tr>
<td></td>
<td>Infants (7 months to 1 year)</td>
<td>11 mg</td>
</tr>
<tr>
<td></td>
<td>Infants (birth to 6 months)</td>
<td>0.27 mg</td>
</tr>
</tbody>
</table>

Table 3: Recommended daily intake of iron in Nordic countries (Norway, Denmark, Sweden, Finland, Iceland)

<table>
<thead>
<tr>
<th>Age</th>
<th>Recommended daily intake in mg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Children</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;6 months</td>
<td>0</td>
</tr>
<tr>
<td>6 months-5 years</td>
<td>8</td>
</tr>
<tr>
<td>6-9 years</td>
<td>9</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
</tr>
<tr>
<td>10-17 years</td>
<td>11</td>
</tr>
<tr>
<td>18-74 years</td>
<td>9</td>
</tr>
<tr>
<td>&gt;75 years</td>
<td>9</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
</tr>
<tr>
<td>10-13 years</td>
<td>11</td>
</tr>
<tr>
<td>14-60 years</td>
<td>15</td>
</tr>
<tr>
<td>61-74 years</td>
<td>9</td>
</tr>
<tr>
<td>&gt;75 years</td>
<td>9</td>
</tr>
<tr>
<td><strong>Lactating</strong></td>
<td>15</td>
</tr>
</tbody>
</table>
Table 4: Recommended daily intake of iron in Finland

<table>
<thead>
<tr>
<th>Age</th>
<th>Recommended daily intake in mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6 months</td>
<td>5</td>
</tr>
<tr>
<td>6 months- 6 years</td>
<td>8</td>
</tr>
<tr>
<td>7-10 years</td>
<td>10</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
</tr>
<tr>
<td>11-18 years</td>
<td>12</td>
</tr>
<tr>
<td>19-75 years</td>
<td>10</td>
</tr>
<tr>
<td>&gt;75 years</td>
<td>10</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
</tr>
<tr>
<td>11-60 years</td>
<td>12</td>
</tr>
<tr>
<td>61-75 years</td>
<td>10</td>
</tr>
<tr>
<td>&gt;75 years</td>
<td>10</td>
</tr>
<tr>
<td><strong>Lactating</strong></td>
<td>12</td>
</tr>
</tbody>
</table>

Source: Finnish Nutrition recommendations, National Nutrition Council committee report 1999, published by the ministry of agriculture and forestry

Iron balance during pregnancy requires iron balance to be 500mg at the start of pregnancy. In the second and third trimesters most women may need iron supplementation.
2.3 Absorption of Iron

Iron is mostly absorbed in the duodenum. The amount of iron absorbed from a typical diet is small and this is also tightly regulated to match the body’s need. Several factors seem to influence this; they include the body iron store, level of erythropoietic activity in the bone marrow, blood hemoglobin concentration, blood oxygen concentration and the presence or absence of inflammatory cytokines (Miret et al. 2003). Therefore the body tends to absorb more iron when the body iron concentration is low; there is increased hematopoiesis for example during pregnancy or during hypoxic conditions. The body tends to absorb less during inflammation or when body iron concentration is high. Derangement in this control leads to iron deficiency anemia or iron overload syndrome respectively.

The cells lining the duodenum are responsible for absorption with the cells lining the villi close to the gastro duodenal junction responsible for almost all the absorption. The Iron must pass from the gut lumen through the enterocytes then out from the basolateral membrane in to the plasma. This process usually consists of about 4 steps (Andrews et al, 1999, Fleming et al, 2005). These are: Reduction of iron from the ferric state (Fe³+) to the ferrous state(Fe²+) by means of the enzyme ferric reductase present on the enterocytes, Apical uptake via Divalent metal transporter 1(DMT1), Intracellular storage as ferritin or trans cellular trafficking, Basolateral release via ferropiortin that requires an accessory protein called hephaestin a copper containing protein similar to ceruloplasmin.

As previously stated, the absorption of dietary iron is tightly controlled to match the body’s needs as there is no definite route or excretion especially in men and post-menopausal women. Pre-menopausal women usually lose more iron during their menstrual cycles.

The absorption of iron is controlled to a variable extent by the amount of recently ingested dietary iron this is the so-called dietary regulator or mucosal block (Hahn et al. 1943). The enterocytes lining the duodenum sense they no longer need to absorb more iron after a meal. This control is insufficient because the body might be deficient for example in the recuperating phases of iron deficiency anemia. Iron absorption is also controlled to a limited extent by total body iron content rather than dietary iron this is the so called stores regulator (Finch, 1994). The molecular mechanisms of this control are not fully understood.

Iron absorption is also controlled by demands for erythropoiesis this is called the erythropoiesis regulator; this probably involves a soluble signal carried by plasma from bone marrow (Finch, 1994)
2.4 Iron transport and storage

The absorbed iron is usually bound to transferrin in plasma for transport to sites of storage or use commonly to the bone marrow for erythropoiesis. Iron is stored as ferritin, a water soluble complex of iron and protoporphyrin. Iron is also stored as hemosiderin a water insoluble complex of iron and protein, it’s usually found in the liver, macrophages in bone marrow and the spleen. A hallmark of iron overload syndromes such as hemochromatosis is the abundance of hemosiderin deposits all over the body especially skin (bronze diabetes) and the liver.

Hepatocytes absorb the iron via transferrin receptor 1 and 2. Hepcidin is an iron regulatory peptide hormone which mediates its effect by affecting the ability of ferroportin to release iron from enterocytes and hepatocytes.

2.5 Measurement of body iron.

There are quite a number of parameters that can be used to assess body iron. Most of these tests can be interpreted together to get a clearer picture. They include serum iron, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), transferrin saturation, ferritin, hemoglobin and hematocrit estimation, zinc protoporphyrin and HFE gene test.

Serum iron just simply measures the amount of iron in blood sample. The reference range is 13-32 µmol/l (Kumar, 2012)

Total iron binding capacity (TIBC): This test measures the amount of proteins that are readily available to bind iron. This is an indirect assessment of transferrin since most of the bound iron in plasma is bound by transferrin. There is thus an inverse relationship between serum transferrin and body iron stores (Fauci et al. 2008). TIBC values 250-370 µg/dl (45-66 µmol/l) (Fauci et al. 2008)

Unsaturated iron binding capacity (UIBC): This is also an indirect assessment of transferrin, usually two-thirds of the binding sites of transferrin are unbound and the amount of saturation increases with increasing iron levels (Fauci et al. 2008).

Ferritin: is the main storage of form of iron and its level closely reflects body stores. Reference value in male is 20-250 µg/L, female 15-150 µg/L (Fauci et al. 2008).
Hemoglobin and hematocrit estimation: These tests are done as part of a complete blood count and there exists a 3:1 ratio between hemoglobin and hematocrit, therefore hemoglobin of 10 g/dl would be a hematocrit of 30%. The reference range for hemoglobin is 13.5 - 17.5 (males) and 11.5 – 16 (females), while for hematocrit it is 40 - 54 % (males) and 37 - 47 % (females) (Kumar, 2012).

Zinc protoporphyrin estimation: protoporphyrin is a precursor to porphyrin, in the absence of iron other metals such as zinc bind to it and if there are increased levels of zinc protoporphyrin it might signify iron deficiency.

HFE gene test: is a test that identifies patients with hereditary hemochromatosis and as such is not a direct estimate of body iron stores. Most patients have a unique missense mutation (C282Y) that alters a major histocompatibility complex (MHC) class 1 like protein called HFE affecting its function with the ultimate result of these patients absorbing 2 or 3 times as much iron as normal people (Feder et al, 1996).

Hepcidin: is a peptide hormone produced by the liver that regulates iron homeostasis. Hepcidin inhibits iron transport across the gut mucosa preventing excessive absorption. It also inhibits the transport of iron out of macrophages where iron is stored, thereby trapping iron and preventing release into the blood. (Andrews, 1999)
2.6 GLUCOSE HOMEOSTASIS

Glucose has been termed the fuel of life. This is because glucose is essential for almost all essential reactions in the body. Glucose is both synthesized and ingested by humans, and its regulation involves a number of hormones secreted by various organs in the body.

![Chemical structure of glucose](image)

**Figure 1** - Chemical structure of glucose


Glucose is a monosaccharide and can exist freely on its own or combined with other monosaccharide units such as fructose or galactose to form other compounds. Glucose is also a component of higher compounds like glycogen. It is found abundantly in many food sources like grains, fruits and vegetables, dairy products and refined sugars. The normal reference value for blood glucose is 70-120 mg/dl (3.9 - 6.7 mmol/l) (Kumar, 2012)
Figure 2: Major organs involved in glucose homeostasis


The liver and pancreas as shown above are the main regulatory organs that control the metabolism of glucose. The liver through a variety of biochemical reactions releases and stores glucose according to body’s needs. The pancreas secretes the major hormones that control glucose homeostasis-insulin, glucagon and many others.

The kidneys also have the capacity to produce small quantities of glucose under normal condition; however this secretion is increased during periods of starvation. The kidneys also filter and reabsorb almost all the body’s glucose, it is essential this reabsorption occurs in order for the body to conserve its glucose store. During periods of glucose excess like in diabetes the capacity of the kidneys to reabsorb glucose is exceeded and glucose is therefore lost in the urine.

Skeletal muscles do not secrete glucose, but have a high capacity to mop up or absorb free glucose in the plasma and store them as glycogen. This is particularly useful in periods of glucose excess. Skeletal muscles however can provide amino acids which serve as building blocks for glucose synthesis in the liver.
The brain utilizes glucose for energy; the body tries to maintain this supply as the brain has very little glucose storage. Brain function begins to deteriorate in periods of low glucose or starvation as the body tries to find alternate energy sources like ketone bodies.

Adipose tissue stores lipids which can be converted to substrates for glucose production by the liver.

### 2.6.1 Intermediary Metabolism

This is a series of interconnected biochemical reactions in the body that maintains glucose homeostasis. Glycolysis is the breakdown of glucose to energy sources like pyruvate with the corresponding production of ATP. Gluconeogenesis is the production of glucose from substrates like alanine which can be derived from protein or fatty acid breakdown. Glycogenesis is the production of glycogen usually from glucose, this is important in the liver and also skeletal muscles in periods of glucose excess. Glycogenolysis is the breakdown of glycogen to produce glucose, essential in periods of glucose lack. (Stryer, 1995)

All these reactions are interconnected with Insulin and glucagon playing essentially antagonistic roles at various rate limiting steps in the cycle.

### 2.62 Insulin

Insulin is produced by beta cells in the islets of Langerhans found in the pancreas. Insulin is a polypeptide produced in a pre-pro form that is then cleaved enzymatically to produce the pro form and finally the hormone (51 amino acids) itself which consists of an alpha and beta chain connected by di-sulfide bonds and C peptide (Fauci et al. 2008).

The pancreas secretes insulin in response to a variety of stimulus principal of which is high glucose levels.

Measurement of insulin therefore is a good indicator of glucose homeostasis as the body secretes insulin in periods of glucose excess to mop up glucose from the body and store it. Insulin does this by stimulating a series of biochemical reactions such as glycolysis and glycogenesis and inhibiting glycogenolysis and gluconeogenesis, the end results of this is to reduce blood glucose levels.
The typical blood level of insulin between meals is 8-11 IU/ml (57 - 79 pmol/l) (Iwase et al. 2011), the half-life of insulin is about 5 minutes (Duckworth et al. 1998), as it is rapidly degraded by the liver and kidney. The accompanying C peptide has a longer half-life than insulin and it’s measured at times to indirectly give an estimate of pancreatic beta cell activity. There are other markers to assess pancreatic beta cell activity and this would be discussed later.

Insulin also promotes fatty acid and protein synthesis in adipose tissue and skeletal muscle and inhibits enzymes that favor breakdown of lipids and proteins. Therefore insulin is seen as a hormone of surplus or boom. It acts to store glucose (energy currency) for periods of lack.

2.63 Glucagon

Glucagon is another hormone secreted by alpha cells of the pancreas also as a pro-hormone that needs to be cleaved to its active form enzymatically (Fauci et al. 2008). The release of glucagon is stimulated by several factors chief of which is low plasma glucose. Glucagon acts to directly oppose insulin at various biochemical reactions. Glucagon therefore stimulates Glycogenolysis and gluconeogenesis while inhibiting glycolysis and glycogenesis. Measurement of serum glucagon while present is not routinely used as a measure of glucose homeostasis.

A couple of other hormones play a role in glucose homeostasis including somatostatin released by delta cells of the islets of Langerhans. Somatostatin is also secreted as a pro-hormone, it acts to inhibit insulin. The counter regulatory hormones such as epinephrine, norepinephrine, cortisol growth hormone all act to raise plasma glucose levels thereby opposing the action of insulin in a couple of these biochemical reactions mentioned earlier.
**Table 5: Summary of the hormonal regulation of glucose homeostasis**

<table>
<thead>
<tr>
<th>Organs</th>
<th>Insulin</th>
<th>Glucagon</th>
<th>Epinephrine</th>
<th>Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycogen breakdown (Glycogenolysis)</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Glycogen synthesis (Glycogenesis)</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Gluconeogenesis</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Glycolysis</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>Glucose release</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Glucose uptake</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td><strong>Pancreas</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin release</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Glucagon release</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td></td>
</tr>
</tbody>
</table>

↑ = Stimulates, ↓ = Inhibits.

### 2.64 Derangements of glucose homeostasis

There are a couple of disease conditions that can results with alteration in the control of glucose metabolism. The most frequently seen are abnormalities in hormonal secretion and enzyme deficiencies.

The hormonal deficiencies commonly seen are deficiencies of insulin and glucagon, there could also be over secretion of these hormones, all leading to various clinical states.

The enzymes mentioned as rate determining steps in intermediary metabolism especially in the glycolytic pathway could also lead to various clinical syndromes. A few of these conditions are discussed below
2.64 Hyperglycemic states

**Diabetes Mellitus**-Diabetes is defined as elevated serum plasma glucose above the reference range usually due to relative or apparent lack of insulin or lack of response of tissues to available insulin. The reference range usually chosen is a random glucose concentration above 200 mg/dl (11.1 mmol/l) plus symptoms of hyperglycemia, a fasting plasma glucose concentration above 126 mg/dl (7.0 mmol/l) two hours post 75 g oral glucose load of greater than 200 mg/dl (11.1 mmol/l), glycated hemoglobin > 6.5% (WHO, 1999).

The typical symptoms of hyperglycemia are polyuria, polydipsia and polyphagia, though these are not usually present in all cases and a few patients may present with complications such as diabetic ketoacidosis, ulcers, gangrene, recurrent vaginal yeast infections and poor wound healing.

Diabetes is usually classified into type 1 and type 2. Type 1 diabetes is the condition where there is usually a gradual decline in insulin secretion with eventual lack of insulin production by the beta cells of the islets of Langerhans. Type 1 diabetes tends to be more common among younger children and its etiology is closely tied with an autoimmune basis (Fauci et al. 2008).

Type 2 diabetes mellitus however is where there is loss of tissue sensitivity to insulin; the beta cells produce insulin but there is end organ failure to utilize the secreted insulin as a result there is an apparent lack in the midst of plenty. Type 2 diabetes mellitus is more common among older people (Fauci et al. 2008).

Glucagonoma is also a relatively less frequent cause of hyperglycemia wherein there is an unregulated production of glucagon by the pancreas resulting in an antagonistic effect on insulin with antecedent hyperglycemia.

**Metabolic syndrome**: This is a constellation of insulin resistance, glucose intolerance, dyslipidemia, hypertension and obesity. There seems to be an underlying etiology common to all these manifestations.

**Impaired/abnormal glucose tolerance**-This is also a relatively common condition that is thought of as a pre-diabetic condition, as a reasonable proportion of patients with this condition eventually develop type 2 diabetes mellitus. Impaired glucose tolerance is associated with insulin sensitivity and is defined as plasma glucose between 140-199 mg/dl (7.8 - 11.0 mmol/l) measured 2 hours after a 75 g oral glucose load (WHO, 1999).
2.642 Hypoglycemic states

Hypoglycemia is defined loosely as low blood sugar, but more strictly defined by the Whipples triad (Cryer et al. 2009) of

1. Symptoms of hypoglycemia like confusion, dizziness sweating

2. Low blood sugar 60-70 mg/dl (3.3 - 3.9 mmol/l)

3. Resolution of the symptoms when blood glucose increases.

Hypoglycemia could be due to a variety of causes from inadequate intake, starvation to insulin producing tumor (insulinoma) and excessive exogenous insulin injection seen in patients with Munchhausen syndrome.

2.65 Assessment of glucose homeostasis

Glucose homeostasis as discussed earlier is tightly controlled and derangements have untoward consequences. It is there essential that measurement of glucose homeostasis is readily available especially for high risk people.

There are several methods of assessing glucose homeostasis and we shall touch on a few.

**Serum glucose** measurements seems to be a fairly easy and reliable assessment of glucose balance, serum glucose is expected to be high in hyperglycemic states such impaired glucose tolerance and diabetes mellitus and low in hypoglycemic states. Serum glucose ranges between 4.4 - 6.1 mmol/L (82 - 110 mg/dl) (Fauci et al. 2008).

**Serum insulin**-This is also a good assessment of glucose homeostasis but as previously mentioned insulin has a short half-life (9) and a such might not provide a reliable estimate of present condition, this could be enhanced with measurement of C peptide which has a longer half-life than insulin (9). Serum insulin ranges between 8 - 11µIU/ml (57 – 79 pmol/l) (Iwase et al. 2001).

**Glycosylated hemoglobin**-This is a test used to monitor diabetic patients. Glycosylated hemoglobin provides a better assay of glucose control as it measures glycemic control of the
preceding 3 months and as such is a more reliable estimate of longer term control. Glycosylated hemoglobin normally ranges between 4 – 5.9 % (20 – 40 mmol/L) (WHO, 1999).

**HOMA**-The homeostasis model assessment estimates beta cell function and insulin sensitivity as a percentage of a normal reference population. This model was first described by David Mathews and his colleagues and was run as a computer program (Matthews et al. 1985), it was subsequently modified by Jonathan levy and colleagues in 1998 (Levy et al 1998).

The model is based on the premise that fasting plasma insulin and serum glucose were determined in part by a hepatic beta cell loop, therefore the increased glucose reflects a compensatory mechanism that maintained fasting insulin levels when there was a reduction in beta cell secretory capacity and secondly that fasting insulin levels were elevated in direct proportion to decreased insulin sensitivity (Turner et al. 1979).

### 2.7 Role of Iron in glucose homeostasis

Elevated body iron has been associated with abnormalities of glucose homeostasis in several studies (Tuomainen, 1997, Haap, 2003, Ford, 1999, Salonen et al. 1998, Jiang, 2004). The exact pathophysiology of the association is not known, but several theories have been proposed.

It is believed that iron has the ability to generate free radicals (Cooksey. 2004, Wolff, 1993), which then cause oxidative damage to tissues (Nancy, 1999, Wolff, 1993, Beard 2001). Iron is essential for a number of biochemical reactions in the body including the electron transport chain, gene regulation, regulation of cell growth and differentiation. Abnormalities in these processes may then explain the results of the manifestation of iron derangements at the cellular level.

The role of iron in the electron transport chain has been postulated to be a major pathway of tissue damage. Iron in excess generates free radicals which have the ability to damage lipids found in cell membranes, damage proteins and also deoxyribonucleic acid (DNA), all these ultimately lead to cell death (Beard 2001), which when it occurs in the beta cells ultimately lead to the development of abnormalities of glucose homeostasis and end result of diabetes mellitus.
Iron when in excess in the body decreases muscle uptake of glucose as demonstrated by Merkel at-al (Merkel et al.1988), in their research with thalassemia patients receiving blood transfusion. Iron is also thought to impair insulin secretion by the beta cells of the pancreas, initial phase of insulin resistance with increased secretion and finally decreased secretion (Wilson et al.2003). Iron reduces insulin extraction by the liver in patients with hemochromatosis (Niederau, 1984).

The exact timeline of the association between body iron and glucose homeostasis is an interesting one. The question of which occurs first has been asked severally. Most authors tend to agree that elevation in body iron tends to occur before clinical manifestations of abnormalities of glucose balance (Salonen et al. 1998, Jiang, 2004, Forouhi 2007, Fumeron 2006), however abnormalities in glucose homeostasis has also been implicated in iron metabolism. Insulin via the activation of hypoxia-inducible factor1alpha may decrease the synthesis of hepcidin, which is a key regulatory enzyme previously mentioned in iron absorption, this leads to uncontrolled absorption of iron with consequent iron overload (McCarty, 2003, Aso, 2010, Le, 2007, Fernandez-Real, 2002).

It is therefore obvious from the above discussion that body iron influences glucose balance along a spectrum from abnormal glucose balance to diabetes mellitus to the metabolic syndrome. It therefore begs to question if by manipulating body iron stores we can observe corresponding changes in indices of glucose homeostasis. Blood transfusion and iron supplementation are methods of boosting body iron stores while blood donation is a method of reducing body iron stores. We wish to see if any of these interventions would result in a corresponding change in glucose homeostasis.

Jose Manuel Fernandez-Real et all concluded in their study of 181 healthy Caucasian men that those who were regular blood donors defined as those with two or more blood donations in the last 5 years had a statistically significant increased insulin sensitivity [3.42 (1.03) vs. 2.45 (1.2) × 10−4 · min−1 · mIU/L; P = 0.04], and decreased insulin secretion [186 (82) vs. 401.7 (254) mIU/L · min; P <0.0001], compared to non-donors. This association also clearly correlated with reduced serum ferritin in the blood donor group. Serum ferritin, 101.5 (74) vs. 162 (100) μg/L; P = 0.017] (Fernandez-Real, 2005)

A study by Ascherio et al. examined the association between blood donation and risk of cardiovascular disease CVD (Ascherio et al. 2001), incidentally found that diabetes mellitus was rarer among the blood donor group as compared with the non-donor group, though this
study did not assess other parameters like insulin resistance. It can therefore be hypothesized from the above that among healthy subjects blood donation resulted in lower serum ferritin levels which resulted in lower risk of abnormal glucose balance.

A study by Hua et al in which 60 patients were divided into two groups of vegetarians (those who reported no meat consumption in the last 5 years) and non-vegetarians (those who ate meat at least once daily in the last 5 years) found that vegetarians were more insulin sensitive than non-vegetarians as assessed by steady state plasma glucose (SSPG) 4.1 vs. 6.9; $P = 0.0028$. (Hua, 2001), among the non-vegetarians 6 patients were selected to undergo monthly or bi monthly phlebotomies of 500 ml of blood, SSPG reduced from 8.8 mmol/l to 5.2 mmol/l; $P = 0.0008$ (Hua, 2001). Though the sample size selected (6 persons) was small they were able to demonstrate the beneficial effects of blood donation on glucose balance parameters.

Among diabetic patients Jose Manuel Fernandez-Real et al, concluded that patients assigned to blood donation groups, those who had 2 phlebotomies of 500 ml each at 2 week intervals compared to non-donors followed over a 12 month period had statistically significant improvements in glucose homeostasis parameters. A statistically significant increase in insulin sensitivity was observed in the blood-letting group (from $2.30 \pm 1.81$ to $3.08 \pm 2.55$ mg/dl/min at 4 months, to $3.16 \pm 1.85$ mg/dl/min at 12 months; $P = 0.045$) in contrast with group 2 subjects (from $3.24 \pm 1.9$ to $3.26 \pm 2.05$ mg/dl/min at 4 months, to $2.31 \pm 1.35$ mg/dl/min at 12 months) (Fernandez-Real et al. 2002). Among patients with fatty liver disease blood donation resulted in significantly lower blood ferritin levels and reduced insulin sensitivity as assessed by HOMA ($P = 0.0016$ for insulin, $P = 0.0042$ for HOMA-R compared to patients on nutritional advice alone (Valenti, 2007).

Iron supplementation was found to be associated with significant increases in insulin resistance (HOMA-IR) among pregnant women (Bo, 2009), it remains to be seen whether this association holds same for men and non-pregnant women. In a large prospective study of men by Jian et-al, heme iron intake from red meat was positively associated with increased risk of DM (RR: 1.63 ,P for trend < 0.001), while the relative risk of diabetes mellitus with frequency of blood donations was not statistically significant (Jiang, Jan 2004). The author then concluded that blood donations are not associated with risk of diabetes mellitus. While this study measured its outcome of frank diabetes by self-reporting of symptoms, diagnosis or treatment of diabetes mellitus during biennial follow-up, there is no data to assess the
influence of blood donations on other parameters of glucose homeostasis such as HOMA before overt diabetes develops. Shafer demonstrated abnormal glucose tolerance in a small cohort of non-thalassemic adults who were transfused over a period of time. The abnormal glucose indices measured included insulin output.

3 Significance of the study

As concluded by both Hua (Hua et al., 2001), and Jose Manuel Fernandez et-al. (Fernandez et-al. 2005) that blood donation is associated with increased insulin sensitivity as assessed by SSPG and the frequently sampled intravenous glucose tolerance test respectively and decreased iron stores, we intend to study this association (body iron and glucose homeostasis) within our cohort of Finish men and women in the KIHD study group. To see if this association holds true using the HOMA as an outcome measurement of insulin resistance and sensitivity so as to identify patients who are having glucose balance abnormalities even before clinical symptoms appear.

In a small study sample of 10 persons studied 4 weeks after a 500 ml phlebotomy by Facchini at al., serum concentrations of ferritin were markedly reduced by 50% (75 ±18 to 38 ±10 μg/l) compared to baseline, after a 75 g glucose load 2 hour plasma insulin was reduced by 37 ± 9% (665 ± 158 to 418 ± 93 pmol/l; P < 0.02) and glucose concentration was reduced by 19 ± 3% (7.4 ± 1.2 to 6.0 ± 0.8 mmol/l; P < 0.05) (Facchini et al. 1998). They demonstrated from their study immediate beneficial effect of blood donation on glucose balance as assessed by blood glucose levels and insulin concentrations. We likewise intend to use a fairly larger sample size that is followed prospectively over an 11 year period to see if these same associations follow can also be demonstrated.

If significant associations are found, it may become imperative to closely look again at benefits of blood transfusion among health subjects, but also among subjects at risk of abnormal glucose balance and those with a positive family history of diabetes mellitus. Our study intends to investigate if the risk of abnormal glucose balance is lower among blood donors as compared to non-donors and as such propose the benefit of more blood donation.

Abnormal glucose balance as a spectrum of disease is highly prevalent in the western societies. In Finland the prevalence of type 2 diabetes mellitus has been on the increasing
trend, among people aged 30 years and above the incidence was 16/1,000 in a survey conducted between 1966-1972, 29/1,000 in 1976 and 32/1,000 in 1980 (Reunanen, 1983).

A study by Saaristo et al. (Saaristo et al, 2008) found that the prevalence of abnormal glucose tolerance was high 42% in men and 33% in women; the high prevalence suggests that undetected glucose imbalance remains high. The findings of this study should therefore have significant public health implications.
**4 AIM:** To study the associations between body iron stores and glucose balance in the KIHD study using the 11-year follow up data.

**Specific objective** - (1) To assess the association between blood donation and glucose balance using HOMA-IR and HOMA-BcF

(2) To examine the association between frequency of blood donation and glucose balance using HOMA-BcF and HOMA-IR.
5 Materials and methods

Study population

The KIHD is a long running prospective (cohort) study designed to investigate risk factors for cardiovascular diseases, atherosclerosis and related outcomes in middle-aged men from eastern Finland. (Tuomainen et al. 1997).

The study population consists of randomly selected men and women living in Kuopio and neighboring rural communities. A total of 2862 men aged 42-60 years were enrolled in the study between 1984 and 1989 and a further 920 post-menopausal women were added from the same area between 1998 to 2001. The 4 year follow up examination was carried out between 1991 and 1993 for men, 11-year examinations between 1998 and 2001 for men and women were then entered into the study population at this time, and 20-year examinations between 2006 and 2008.

Data collection

This study used the 11-year follow up examination data for the analysis. A Total of 1774 were recruited for the study, patients were selected randomly from the 11 year cohort. We excluded patients with diabetes (n=213) from the study as defined by WHO criteria- fasting plasma glucose > 7mmol/L (126mg/dl) or 2hour post-prandial glucose > 11.1 mmol/L (200mg/dl) (WHO 1999), those who had missing data on history of blood transfusion (n=6), leaving a total of 1555 for the current analysis.

Blood donation was assessed by data linkage from the records of the local Red Cross office. In each donation 500 mls of blood was collected.

Glucose homeostasis was assessed by measuring the HOMA-IR and BcF as explained previously on page 27.

Measurements

Questionnaires were administered during the 11-year follow up to assess family history of diabetes, hypertension, drug use for diabetes and hypertension, smoking status and number of cigarettes smoked. Physical examination was also conducted during these follow-up examination and physiological parameters like blood pressure was assessed. (Salonen et al. 1992)
Blood was collected by phlebotomy to assess serum insulin, blood glucose levels and lipid profiles—serum HDL, LDL and triglycerides. (Nyyssonen et al. 1996)

5.1 STATISTICAL ANALYSIS

The baseline characteristics of the study population are presented as mean (± SD) according to serum ferritin quartiles. The test of linear trend were conducted by assessing the median values for each category or exposure variable and treating those as continuous variable.

The Linear regression model was used to assess the association between blood donation ever and HOMA-IR and HOMA-BcF

Further analysis was done to show the association between the frequency of blood donation (at least 2 blood donations in a year) and HOMA-IR and HOMA-BcF.

The analysis was done using SPSS version 21 for windows. All p values were two tailed and < 0.05 was considered statistically significant.

6 ETHICAL CONSIDERATION

Approval was requested from the ethics committee of the university and the data was assessed on the 14th of November 2013.
Table 6: Baseline characteristics of study population according to quartiles of serum ferritin

<table>
<thead>
<tr>
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<th>Serum ferritin quartiles µg/L</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td></td>
<td>n=2-34</td>
<td>n=35-68</td>
</tr>
<tr>
<td>Serum insulin (mU/L)</td>
<td>7.25 ± 4.78</td>
<td>8.22 ± 16.47</td>
</tr>
<tr>
<td>Blood glucose (mmol/L)</td>
<td>4.74 ± 0.43</td>
<td>4.76 ± 0.43</td>
</tr>
<tr>
<td>Diabetes in family (yes/no)</td>
<td>3.6</td>
<td>3.2</td>
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<tr>
<td>Hypertension in family (yes/no)</td>
<td>5.7</td>
<td>5.9</td>
</tr>
<tr>
<td>Drug for hypertension (yes/no)</td>
<td>3.37</td>
<td>4.04</td>
</tr>
<tr>
<td>Drug for cholesterol (yes/no)</td>
<td>0.039</td>
<td>0.039</td>
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<tr>
<td>Mean systolic blood pressure (mmHg)</td>
<td>136 ± 17</td>
<td>135 ± 18</td>
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<tr>
<td>Mean diastolic blood pressure (mmHg)</td>
<td>80 ± 9</td>
<td>80 ± 9</td>
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<tr>
<td>Serum triglycerides (mmol/L)</td>
<td>1.16 ± 0.53</td>
<td>1.09 ± 0.49</td>
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<tr>
<td>Serum HDL (mmol/L)</td>
<td>1.30 ± 0.31</td>
<td>1.29 ± 0.30</td>
</tr>
<tr>
<td>Serum LDL (mmol/L)</td>
<td>3.55 ± 0.88</td>
<td>3.60 ± 0.89</td>
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<td>Body mass Index (kg/m²)</td>
<td>27.09 ± 4.29</td>
<td>27.18 ± 4.62</td>
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<tr>
<td>Waist to hip ratio</td>
<td>0.87</td>
<td>0.88</td>
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<tr>
<td>Smoker (%)</td>
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<td>12</td>
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<tr>
<td>Number of cigarettes smoked per day</td>
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<td>1.51</td>
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<tr>
<td>Amount of alcohol consumed per week (grams)</td>
<td>32</td>
<td>27</td>
</tr>
<tr>
<td>Gender % (Male)</td>
<td>29.5</td>
<td>36</td>
</tr>
<tr>
<td>Mean age in years</td>
<td>62.99 ± 6.53</td>
<td>63.01 ± 6.59</td>
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</table>
Table 7: Linear regression analysis between HOMA-IR and blood donation ever

<table>
<thead>
<tr>
<th>Co-variates</th>
<th>Standard coefficients</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donation ever</td>
<td>0.049</td>
<td>0.024</td>
</tr>
<tr>
<td>Mean age in years</td>
<td>0.078</td>
<td>0.001</td>
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<tr>
<td>Gender</td>
<td>0.044</td>
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<tr>
<td>Date of examination</td>
<td>0.022</td>
<td>0.309</td>
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<tr>
<td>Body mass index</td>
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<tr>
<td>Smoker</td>
<td>0.036</td>
<td>0.32</td>
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<tr>
<td>number of cigarettes per day</td>
<td>0.006</td>
<td>0.87</td>
</tr>
<tr>
<td>Alcohol, (gram/week)</td>
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<td>0.675</td>
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<td>Diabetes in family</td>
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<td>0.236</td>
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<tr>
<td>Hypertension in family</td>
<td>0.019</td>
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<tr>
<td>Drug for high cholesterol</td>
<td>0</td>
<td>0.999</td>
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<tr>
<td>Mean systolic blood pressure</td>
<td>0.006</td>
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<tr>
<td>Mean diastolic blood pressure</td>
<td>0.013</td>
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<tr>
<td>Serum triglycerides</td>
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<td>Serum HDL</td>
<td>0.057</td>
<td>0.021</td>
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<td>Serum LDL</td>
<td>0.033</td>
<td>0.126</td>
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<tr>
<td>Rank of ferritin</td>
<td>0.057</td>
<td>0.011</td>
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Table 8: Linear regression analysis between HOMA-beta cell function and blood donation ever

<table>
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<tr>
<th>Co-variates</th>
<th>Standard coefficients beta</th>
<th>significance</th>
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<tr>
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<td>0.406</td>
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<tr>
<td>Mean age in years</td>
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<td>Gender</td>
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<td>Body mass index</td>
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<td>number of cigarettes per day</td>
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<td>Drug for high cholesterol</td>
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<td>Mean systolic blood pressure</td>
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<td>0.958</td>
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<td>Mean diastolic blood pressure</td>
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<td>Serum triglycerides</td>
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<td>Serum HDL</td>
<td>0.078</td>
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<td>Serum LDL</td>
<td>0.038</td>
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<tr>
<td>Rank of ferritin</td>
<td>0.025</td>
<td>0.313</td>
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</table>
7 RESULTS

The baseline characteristics of the study population is shown in table 6.

The mean age was 62.73 ± 6.5, while the mean serum ferritin was 97.13 µg/L.

Individuals who had higher serum ferritin were more likely to have higher serum insulin, higher blood glucose levels, higher diastolic blood pressures and higher triglycerides.

Individuals with lower serum ferritin had lower, LDL higher HDL. Lower BMI, waist to hip ratio smoked less cigarettes and consumed less alcohol than individuals in the higher serum ferritin quartiles.

In the analysis between blood donation ever and HOMA-IR an inverse relationship (β = -0.049, P= 0.024) was observed between history of blood donation and HOMA-IR. Other statistically significant predictors of HOMA-IR included age in years, body mass index, serum triglycerides and serum ferritin. (Table 7)

There was also an inverse relationship between blood donation ever and HOMA-BcF (β = -0.020, P = 0.406) other statistically significant predictors in the model included body mass index, serum triglycerides and serum HDL. (Table 8)

In the analysis of the association between frequency of blood donation (at least 2 donations per year) and HOMA-IR and HOMA-BcF a non-statistically significant response was observed in HOMA-BcF. (β = 0.046, P = 0.770) and a statistically significant direct association in HOMA-IR (β = 0.280, P=0.021).
8 DISCUSSION

The study examined the association between blood donation as assessed by blood donation ever and frequency of donation, and glucose homeostasis as assessed by the HOMA-IR and BcF.

Our results showed that there is an inverse association between blood donation ever and insulin resistance. Similar association was observed with beta cell function, but was not statistically significant.

This is to be expected as lower serum ferritin has previously been shown to be associated with increased insulin sensitivity and reduced insulin secretion (Fernandez, 2005).

In a study by Khosrow et al, in which the effects of phlebotomy induced reduction of body iron stores on metabolic syndrome was investigated, 64 patients were randomly assigned into iron reduction (N=33) and control groups (N=31), the phlebotomy group had removal of two volumes of blood at entry and at day 28, while both groups had blood collected for final analysis at 6 weeks, they found a reduction in the HOMA index Insulin sensitivity from 4.8 ± 7.2 to 3.6 ± 2.7 in the iron reduction group and from 4.5 ± 3.8 to 4.1 ± 3.6 in the control group giving a group difference of -0.7, P = 0.29.

This is in line with our study though different outcomes were used while we assessed glucose homeostasis by the HOMA 2 for insulin resistance and beta cell function, the study by Khosrow used HOMA index for insulin sensitivity by measuring fasting plasma glucose × serum insulin ÷ 25. Also while we assessed healthy patients without diabetes the study used only patients fulfilling the criteria for the metabolic syndrome (insulin resistance, glucose intolerance, dyslipidemia, hypertension and obesity) this of course is bound to explain differences in results.

The conclusions drawn by Khosrow S et al included the fact that their results on insulin sensitivity were not statistically significant possibly because they had a small sample size, did not remove enough blood from the phlebotomy group as compared to other studies, 550-800ml as compared to 1500mls in the study by Jose Manuel Fernandez et all (Fernandez, 2002).

In another study by Jose Manuel Fernandez et al (Fernandez, 2005) 181 healthy men were randomly assigned into blood donor groups and control groups, they found serum ferritin was
associated with insulin sensitivity \((r = -0.23, P = 0.002)\), further analysis found that the number of blood donation correlated with insulin sensitivity \((r = 0.28, P = 0.01)\).

The findings by Jose Manuel Fernandez et all (Fernandez, 2005) is in line with our findings in this study where we showed that glucose homeostasis as assessed by HOMA-IR where we had a statistically significant correlation when we sub analyzed data from subjects who had 2 or more blood donations a year \((r = 0.28, P = 0.021)\), we however did not have similar results when we assessed glucose homeostasis by HOMA BcF \((r = 0.046, P = 0.770)\), we hypothesize that it is possible we were unable to show a statistically significant result either because of the small number of subjects with significant blood donations or that these subjects already had little or no beta cells left at the time of measurement even though they had not manifested overt clinical signs and symptoms of diabetes.

We had excluded patients with diabetes as defined by the WHO criteria Plasma glucose > 6.9g/dl and or 2hr post-prandial > 11g/dl from our study at the beginning, studies involving diabetics, patients with iron induced insulin resistance and carriers of hemochromatosis gene (Fernandez 2002, Krosrow et al 2012) all showed statistically significant improvements in Hb A1c and HOMA index with phlebotomy or blood donation.

Our study showed a negative association between blood donation and insulin resistance (beta = -0.049, P= 0.024), this is to be expected because lower serum ferritin stores associated with blood donation has been found to be associated with better glucose homeostasis in diabetics, and patients with hemochromatosis (Fernandez 2002, Krosrow et al 2012) as assessed by HbA1c and HOMA index insulin sensitivity. We can then safely conclude that if these reductions are true for patients already with diabetes it should hold true for healthy patients and our results has shown this.

In a study by Chul-Hee at al to study the association between elevated serum ferritin concentration with insulin resistance and impaired glucose metabolism in Korean men and women, 7253 subjects with normal fasting glucose (NFG) , 3783 with impaired fasting glucose( IFG) and 1054 subjects with diabetes mellitus (DM) were assessed the HOMA-IR was for men 1.28 ± 0.80, 2.01 ± 1.20 and 2.88 ± 1.94 for the three groups respectively while for women 1.20 ± 0.71, 2.10 ± 1.14, 3.15 ± 2.31 respectively for the three groups, statistically significant in men but not in women. The HOMA BcF was not significantly correlated with serum ferritin in men and women.
This is in line with our study which showed statistically significant correlation between blood donation ever and HOMA-IR (beta = -0.049, P = 0.024) but did not show an association between blood donation and HOMA BcF (β = -0.020, P = 0.406).

Our study did not show any gender difference between HOMA-IR and BcF when comparing data from men and women, a likely explanation for this is probably because post-menopausal women were used in our cohort and it is generally believed that the body iron stores of these group of women are close to that of men.

The likely reason for not observing a statistically significant association between blood donation ever and HOMA BcF might be due to the fact that the patients included already had reduced or almost non-existent beta cell function without developing overt diabetes at analysis or that the frequency of blood donation in these patients was not enough to show a statistically significant increase in beta cell function.

Beta cell function as assessed by HOMA is not as sensitive as via measurement of C-peptide concentration and failure to show a statistically significant correlation between blood donation and beta cell function might be due to measurement differences.

Furthermore sub analysis of our data to account for frequency of blood donation by analyzing subjects who had at least 2 blood donations in a year showed a statistically significant correlation between blood donation and HOMA-IR (β = 0.280, P = 0.021), but the association was positive though still not statistically significant ( β = 0.046, P = 0.770) for HOMA BcF.

The mechanisms through which iron causes insulin resistance has been explained earlier (Andrews, 1999). Iron is an oxidant that catalyzes redox reactions at the cellular level and has been shown to interfere with insulin signaling at the cellular level (Qian, 1998), also insulin resistance may be the cause rather that the consequence of the disturbances in iron metabolism.

Elevated serum ferritin may reflect inflammation as ferritin is an inflammatory marker, but this cannot be exactly excluded from our study as we did not exclude patients who could have raised inflammatory makers from other disease conditions whether this is an explanation for the association with insulin resistance but not beta cell function is a matter for a further larger study possibly correcting for disease conditions both inflammatory and infectious that can account for raised inflammatory markers such as ferritin.
Regular blood donors are more likely to be healthy than non-blood donors and as such some of the association we saw in our data might be due to this selection bias. It is not unusual to have this scenario as people with healthy lifestyle are more than likely to donate blood and have better glucose homeostasis indices. In a study by Salonen et al (Salonen, September 1998) in which they studies the association between blood donation and myocardial infarction risk, they found that regular blood donors had reduced risk of myocardial infarction as compared to non-donors (RH = 0.058, p = 0.035) (Salonen, September 1998), after correcting for confounding variables the relative hazard increased by 69%. We can therefore hypothesize that in our study the association might be due to this selection bias but based on empirical evidence it could still be statistically significant when these confounders are corrected for.

Blood donation might also lead to a reduction in serum lipids which are confounders for the association between body iron stores and glucose homeostasis. Blood donors have been shown to have lower lipid profiles as shown in table 8, whether this association between blood donation and glucose homeostasis is explained in part through its action on blood lipids remains unclear.
STRENGTHS OF THE STUDY

The study used quite a large sample size 1555 healthy subjects who did not have diabetes at entry and as such it was able to show association between body iron stores and glucose homeostasis even before clinical symptoms and signs of diabetes developed.

The study was a cross sectional study and as such there was no loss to follow-up

LIMITATIONS OF STUDY

The study is a cross sectional study and as such reverse causation cannot be completely ruled out.

While we excluded patients with diabetes from the beginning we did not completely exclude patients who had impaired glucose metabolism such impaired fasting glucose or the metabolic syndrome.
CONCLUSION

The study is congruent with previous studies that have shown that increased serum ferritin is associated with poor glucose homeostasis which ever measurement of outcome is used. The frequency of blood donation is also correlated with better parameters of glucose homeostasis as assessed by HOMA-IR.

It might be more rewarding to conduct a larger study possibly a randomized clinical trial assigning patients into groups of blood donors and non-blood donors and following them up over a period of time to see if this association holds true, we could further sub analyze the blood donor groups by assigning them into well-defined groups based on frequency of blood donation during the follow up period.
10 RECOMMENDATION

Blood donation should be generally encouraged among healthy volunteer’s asides the altruistic benefits, there are personal gains to be achieved including better glucose homeostasis.

The benefit of blood donation are not only evident in patients with iron overload syndromes but are particularly important in healthy subjects and supposedly healthy subjects who might be having impaired fasting glucose or pre-diabetes who haven’t manifested clinical signs and symptoms.
11 REFERENCES


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