Iron Depletion in Rats May Protect Against Atherosclerosis and Ischemic Heart Disease

Fayig El-Migdadi *[1]

Abstract

Experimental evidence cites that iron overload enhances and iron depletion decreases the formation of an atherosclerotic plaque. Many facts seem to support this hypothesis. It helps to explain the striking sex difference in heart disease. In premenopausal women, incidence of atherosclerosis and coronary vascular disease (CVD) is less than half that of age-matched men. Depletion of iron stores by regular menstrual blood loss may be a source of protection in premenopausal patients. Menstruating women maintain the negligible levels of stored iron found in both men and women prior to age 20. Men lacking the menstrual iron 'leak' undergo progressive accumulation of iron rising rapidly with age. At age 45, men have roughly 4 times more iron in storage than women. To test this hypothesis, an animal rat protocol was followed. Two groups (N = 10 for each) of mature adult Sprague-Dawley rats were selected. Basal serum ferritin levels were recorded and the two groups were given one of two diets, a normal chow or a high-iron diet for a period of 4 weeks. Serum ferritin levels were measured at 2 and 4 weeks. Five rats from each group were killed after 2 weeks and the remaining 5 animals after 4 weeks. Blood was collected for a lipid profile and a complete blood count and coronary vessels were examined for atherosclerotic changes. Three serial sections of the coronaries starting from the origin of the arteries were taken and examined histopathologically. In a retrospective study in both human sexes, which was taken from the records of the clinical laboratories in the Eastern Province of Saudi Arabia, there was a significant correlation between coronary artery disease (myocardial infarction) and the higher serum ferritin levels.

Keywords: Iron Depletion, Rats, Humans, Ferritin, Atherosclerosis.

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Introduction

Cardiovascular disease (CVD) is the leading cause of death in developed countries. The established risk factors associated with the disease are:

1. Lifestyle: smoking, diet, excessive alcohol consumption and physical inactivity.
2. Biochemical and physiological: elevated blood pressure and LDL cholesterol, hyperglycemia and diabetes, and obesity.
3. Personal: age, sex, family and personal history. [1]

It has been noted, however that a fair proportion of people with CVD do not have the traditional risk factors. The search is on to find another factor (or factors) that would complete the etiological picture of cardiovascular disease.

One possibility currently being investigated is the association of infectious disease with CVD; either as a risk factor itself or an intensifier of other risk factors.[2] This is not a new idea. Sir William Osler first proposed a causative role for infection in heart disease in 1908.[3]

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Biochemical Basis

The main pathological process underlying both cardiovascular and cerebrovascular diseases is atherosclerosis, or the accumulation of lipid-containing material within the blood vessels, causing arterial occlusion.[2]

Various mechanisms have been offered to support the idea that infection and the inflammatory process affects the arteries and leads to disease onset. One scenario suggests that when bacteria attack the vessel wall, the inflammatory response leads to an increase in lymphocytes and macrophages and in the production of cytokines and tissue growth factors. Tissue growth factors are within the clotting cascade. Lipid metabolism is also regulated during this response; lipoproteins are scavengers of foreign materials such as bacterial endotoxins. Cytokines are a mediator in this response and they also can increase very low-density lipoprotein levels, another risk factor for CVD. [4]

Pesonen et al. [5] found an association between the thickening of the inner coat of blood vessels and infectious disease based on autopsied children. They concluded that infections in general were associated with this thickening and suggested this association may predispose coronary arteries to atherosclerosis.

Repeated fibroproliferative events lead to atherosclerosis.[6] Response-to-injury theory states that various factors, such as infection, can cause these events and damage endothelial cells.

Evidence

The conditions or infectious agents most frequently studied include the Chamydia pneumoniae, Helicobacter pylori, cytomegalovirus, herpes simplex virus and periodontitis. The focus of current research is on the association of C. pneumoniae and atherosclerosis. Evidence for a possible association comes from two types of studies: seroepidemiology and the demonstration of the organism within atherosclerotic tissue.

Saikku et al. [7] found that a chronic Chlamydia infection was higher in patients with acute myocardial infarction and chronic coronary heart disease than in controls. Grayston [8] reported 38 studies in seroepidemiology confirming the association between C. pneumoniae and atherosclerosis. Leinonenn and Saikku [9] suggest that C. pneumoniae interacts with other risk factors of atherosclerosis. On the other hand, Danesh [10] from his meta-analysis of prospective studies concluded no strong evidence to support an association between CHD and C. pneumoniae or any of the other infectious diseases investigated.

So the picture is not complete. The association between cardiovascular disease and infection is biologically plausible; there is some evidence to support an association, but it is inconclusive. There are two other possible pieces to the picture: the so-called iron hypothesis and sex-specificity of infection and CVD.

Iron Hypothesis

The proposal is that iron depletion protects against ischemic heart disease. Experimental evidence was cited [11, 12] that iron overload enhances and iron depletion decreases the formation of atherosclerotic plaque. Various facts support this hypothesis. It helps to explain the striking sex difference in heart disease. In premenopausal women, the incidence of atherosclerosis and CVD is less than half that of age-matched men.

Depletion of iron stores by regular menstrual blood loss may be a source of protection in premenopausal patients. [9] Menstruating women maintain the negligible levels of stored iron found in both men and women prior to age 20. Men lacking the menstrual iron ‘leak’ undergo progressive accumulation of iron rising rapidly with age. At age 45, men have roughly 4 times more iron in storage than women.

One study looked at the association between serum ferritin concentrations and the 5-year progress of carotid atherosclerosis.

The researchers claim their present 'strong' epidemiological evidence for a role of iron stores in early atherogenesis. They further suggest that the promotion of lipid peroxidation is the underlying pathomechanism. Volunteer blood donation has also been associated with a significant decrease in atherosclerosis and vascular events.[1]

The iron hypothesis may help explain the low heart disease rates in third world countries where diets are high in fiber that retards iron absorption. Parasites also cause blood loss from the gut and the bladder. The effect of aspirin on reducing disease rates may be due to the gastrointestinal microbleeding caused by its daily use.[11] Iron was shown to be essential for the development of some microbiotic infections [13], but there was no evidence on whether or not C. pneumoniae is an iron-lover.

Gender Specificity

One curious anomaly persists. Men are more prone to heart disease in early life than women. Are men also more prone to infectious diseases than women?

O'Neill [14] found more males seropositive (for C. pneumoniae) than females (72.9 vs. 67.0). The difference was not found to be statistically significant (chi 2 = 1.6, p. = 0.21). These authors commented that it is 'intriguing to speculate' that a gender difference in infection with C. pneumoniae might contribute to the sex-specific difference in the incidence of cardiovascular disease. They concluded, however, that further investigations are needed to establish a higher prevalence of infection in males.

Almiral [15] looked at the epidemiology of a community-acquired pneumonia. The two most common pathogens are Streptococcus pneumoniae and Chlamydia pneumoniae. Their data show an increase in the incidence rate with age and a higher incidence rate in males than females. (A graph was shown, but no data.) In the original study which isolated C. pneumoniae in acute respiratory tract infections, Grayston et al. [16] found a higher frequency of antibody to the strain in males than females (42% vs. 35%).

Testing the hypothesis regarding iron stores and atherosclerosis and CVD would indeed support the proposed association between CVD and iron metabolism and the sex differences (gender susceptibility to CVD) and infection.

There were 2 specific objectives in this project:

1. To find a correlation between high serum ferritin in high-iron diet fed rats and changes in the lipid profile and atherosclerosis in coronaries.
2. To study retrospectively, from the records of the clinical laboratories in the Eastern Province of Saudi Arabia, the association between coronary artery disease and serum ferritin levels.

Methodology and Experiments

An animal rat protocol was followed. Two groups (N = 10 for each) of mature adult Sprague-Dawley rats were selected. Basal serum ferritin levels were recorded and the two groups were given one of two diets, a normal chow or a high-iron diet for a period of 4 weeks. Serum ferritin levels were measured at 2 and 4 weeks. Five rats from each group were killed after 2 weeks and the remaining 5 animals after 4 weeks.

Blood was collected for a lipid profile and a complete blood count and coronary vessels were examined for atherosclerotic changes. Three serial sections of the coronaries starting from the origin of the arteries were taken and examined histopathologically using hemotoxylin and eosin staining.

Ferritin concentrations in rat serum were measured by the 2-site immunoradiometric assay of Ward et al.[17] Hematocrit (% packed cell volume) was measured by collecting blood from the tail vein into heparinized microhematocrit tubes and centrifuging the tubes in a microhematocrit centrifuge equipped with a reading scale. Hemoglobin concentration was determined by the cyanmethemoglobin method of Crosby et al.[18] Plasma lipids were measured by enzymatic timed-endpoint methods using the

Synchron CX system (Beckman Coulter)[19] and glucose by the glucose oxidase procedure with a Beckman glucose analyser.[20]

A retrospective survey of the association between coronary artery disease and serum ferritin was performed. Records from the clinical laboratories of King Fahd Teaching Hospital in the Eastern Province of Saudi Arabia were taken and subjected to statistical analyses.

Permission was also taken from the appropriate authorities in the clinical laboratories of the above-mentioned hospitals, including the laboratories' directors and the hospital's administrations. This project was also approved by the University Research Committee, the University Human Ethics Committee, and the Deanship of Scientific Research at Jordan University of Science and Technology. All animal work followed the University Procedures for Animal Care and Scientific Research.

All data in the animal protocol and the retrospective survey were subjected to student's *t*-tests and the *p*-value was determined to assess significance between comparable groups. A *p*-value of less than 0.05 was considered significant.

Results and Discussion

A difference in growth between the 2 groups of rats was apparent. Rats eating normal chow grew from 124 ± 8 g to 233 ± 23 g after 4 weeks. On the other hand, rats eating a high-iron diet grew from 128 ± 13g to 278 ± 17 g after 4 weeks. Consumption of food was comparable in the 2 groups (39 - 40 g/2 days for rats eating normal chow, compared to 37 - 38 g/2 days for rats eating a high-iron diet).

Hemoglobin concentration and hematocrit were measured after 2 weeks and after 4 weeks in order to assess iron status. In rats eating normal chow, hemoglobin concentrations grew from 11 ± 2 g/dl to 13 ± 2 g/dl after 2 weeks and to 14 ± 1 after 4 weeks. In rats eating a high-iron diet, hemoglobin concentrations went from 12 ± 2 g/dl to 14 ± 2 g/dl after 2 weeks and to 15 ± 3 after 4 weeks. Changes in hematocrit concentrations in both groups were similar to those changes in hemoglobin concentrations. Changes in both hemoglobin and hematocrit concentrations were all within the normal physiological levels for this type of rat.[21]

Serum ferritin levels in rats eating normal chow (Table 1) showed a significant increase after two weeks and more increase after 4 weeks. The increase in serum ferritin levels, however, was within the normal physiological variations for this type of Sprague-Dawley rats.[21]

Basal serum ferritin levels in rats eating a high-iron diet (Table 2) were comparable to those in rats eating normal chow (Table 1). Serum ferritin levels in rats eating a high-iron diet (Table 2) also showed a significant increase after two weeks and even much more increase after 4 weeks. After 4 weeks, the increase in serum ferritin levels in these rats was much higher than that seen in rats eating normal chow (3.2988 ± 0.450161 versus 2.2188 ± 0.143071). These levels were higher than the normal physiological variations for this type of Sprague-Dawley rats.[19]

After 4 weeks, in the cross-sections of arteries (left anterior descendens coronary artery) taken from the rats eating normal chow, there was no significant arterial lesions observed (Fig. 1). On the other hand, in those cross-sections of rats eating a high-iron diet, there were eccentric intimal thickening and proliferation of smooth muscle cells observed. It is suggested here that iron overload seems to augment the formation of atherosclerotic lesions. Unfortunately, the histopathology data for other cross-sections of the other coronary arteries failed to show any changes and were inconclusive. It is acknowledged here that the limitation of the histopathologic data to those of the left anterior descendens artery, would also limit the extent of our conclusions. Also other vessels were not examined in this study. Future studies should include other vessels including the aorta other major vessels as well as the rest of the coronaries.

After 4 weeks of eating normal chow, rat liver and spleen ferritin concentrations were 150 ± 14 and 141 ± 21 μg ferritin/g tissue, respectively. After 4 weeks of eating a high-iron diet, rat liver and spleen ferritin concentrations were 278 ± 34 and 245 ± 18 μg ferritin/g tissue, respectively.

These data are similar to those previously reported by Hunter [21] and support the notion of a general correlation between the serum ferritin levels and their corresponding liver (and spleen) ferritin concentrations.

Table (1): Serum Ferritin Levels in Rats Eating Normal Chow.

Number of rats in a group	1	2	3	4	5	MEAN±SD
Basal serum ferritin (μg/l)	1.575	1.798	2.157	1.895	2.179	1.9208 ±0.253861
Serum ferritin after 2 weeks (μg/l)	2.146	2.067	2.131	2.2083	2.383	2.1870 ±0.120506
Serum ferritin after 4 weeks (μg/l)	2.233	2.152	2.035	2.249	2.425	2.2188 ±0.143071

Table (2): Serum Ferritin Levels in Rats Eating a High-iron Diet.

Number of rats in a group	1	2	3	4	5	MEAN±SD
Basal serum ferritin (μg/l)	2.237	1.754	2.162	2.293	1.838	2.0568 ±0.244382
Serum ferritin after 2 weeks (μg/l)	3.217	1.847	2.318	2.827	2.59	2.5598 ±0.51739
Serum ferritin after 4 weeks (μg/l)	3.512	2.762	2.93	3.42	3.87	3.2988 ±0.450161

Table (3): Total Cholesterol, HDL-cholesterol and LDL Levels in Rats Eating Normal Chow after 4 Weeks.

Number of rats in a group	1	2	3	4	5	MEAN±SD
Total cholesterol (mg/dl)	113	127	123	138	118	123.8 ± 9.523655
HDL-cholesterol	65	58	52	65	56	59.2 ± 5.718391
LDL	43	48	53	51	48	48.6 ± 3.781534

Table (4): Total Cholesterol, HDL-cholesterol and LDL Levels in Rats Eating A High-iron Diet after 4 Weeks.

Number of rats in a group	1	2	3	4	5	MEAN±SD
Total cholesterol (mg/dl)	154	143	133	164	173	153.4 ± 15.97811
HDL-cholesterol	63	77	69	78	62	69.8 ± 7.52994
LDL	66	58	64	68	66	64.4 ± 3.847077

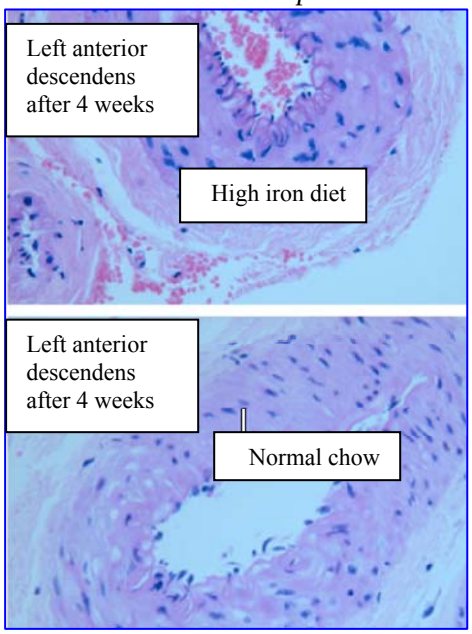

Figure (1): Effect of a high-iron diet and normal chow on rat left anterior descendens coronary arteries. Clear atherosclerotic changes with luminal narrowing are seen with a high-iron diet after 4 weeks. hematoxylin and eosin staining. H and E × 400.

As seen from Tables 3 and 4, after 4 weeks total serum cholesterol levels were higher in rats eating a high-iron diet compared to those levels in rats eating normal chow 123.8 ± 9.523655 mg/dl and 153.4 ± 15.97811 mg/dl. Similarly, HDL-cholesterol levels were also higher in rats eating a high-iron diet (69.8 ± 7.52994 mg/dl compared to 59.2 ± 5.718391 mg/dl). Surprisingly, LDL levels were also higher in rats eating a high-iron diet after 4 weeks (64.4 ± 3.847077 mg/dl compared to 48.6 ± 3.781534 mg/dl). Based on these data, it seems that there is a correlation between the high-iron load and total cholesterol and also both HDL-cholesterol and LDL levels.

More detailed studies are needed to verify other lipid profiles as well as tissue lipid profiles. In fac, there are conflicting data in the literature regarding the lipid profile and the high-iron load and serum ferritin in rats as well as in human subjects.[22-27]

In similar fashion to lipid levels, after 4 weeks blood glucose levels were higher in rats eating a high-iron diet compared to those eating normal chow (142 ± 14 mg/dl versus 89 ± 11 mg/dl).

In 18 cases of myocardial infarction in female Saudi human subjects (mean age of 54 ± 6 years old), serum ferritin levels were 103 ± 39 µg/l. For comparison purposes, these levels were only 66 ± 23 µg/l in 50 female Saudi human subjects (mean age of 24 ± 8 years old), who were selected from the same geographical Eastern Province of Saudi Arabia. These subjects were tested for serum ferritin while seen in the outpatient clinics of King Fahd Teaching Hospital. All subjects in this survey were chosen from subjects tested for serum ferritin at King Fahd Teaching Hospital Clinical Laboratories while they were either patients in the hospital or as outpatients in the clinics. These data were taken in a retrospective survey from the records of the clinical laboratories. In a similar survey, in 21 cases of myocardial infarction in male Saudi human subjects (mean age of 48 ± 12 years old), serum ferritin levels were 237 ± 49 µg/l. Those levels were only 168 ± 37 µg/l in 25 male Saudi human subjects (mean age of 45 ± 8 years old). These data for both human sexes show a clear correlation between myocardial infarction and the higher serum ferritin levels. However, it is too early to overlook all other variables in the acute phase of myocardial infarction.

Many of the acute phase reactants (e.g. WBC, ESR among others) could be affected in these as well as all other subjects having an acute phase of myocardial infarction. The association (and the suggested herein correlation) between myocardial infarction and high ferritin levels can be a causal factor, but one cannot also exclude the possibility of acute phase reaction as well.

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مدى تأثير نقص الحديد في الجرذان في الحماية من تصلب الشرايين وأمراض الشرايين التاجية

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الملخص

أشارت الدراسات العلمية المبنية على التجارب العلمية إلى أن زيادة الحديد في الجسم تزيد من تكوين صفيحة تصلب الشرايين بينما نقصانه في الجسم يقلل من تكوين هذه الصفيحة. تدعم الكثير من الحقائق هذه الفرضية. فهذه الفرضية تساعد على تفسير الفروقات بين الجنسين في أمراض القلب. فعند النساء قبل سن اليأس مدى حدوث تصلب الشرايين وأمراض الشرايين التاجية في القلب لا يزيد عن نصف هذا المدى عند الرجال بنفس عمر هذه الفئة من النساء. قد يكون نقص مخازن الحديد عند النساء في هذه الفترة بسبب فقدان الدم أثناء فترات الحيض هو المسؤول عن هذه الحماية من أمراض تصلب الشرايين وأمراض الشرايين التاجية عندهن.

هنالك نسبة قليلة جداً لا تذكر من الحديد المخزّن عند النساء أثناء فترة الحيض مقارنة بتلك الموجودة عند الرجال وعند النساء قبل البلوغ (أقل من ٢٠ سنة). هنالك نسبة تراكم متزايدة من الحديد عند الرجال مع زيادة العمر وذلك بسبب عدم وجود دم مفقود عندهم بسبب عدم وجود حيض عند الرجال.

في سن ال (٤٥) عاماً، يحتوي جسم الرجل على كمية من الحديد المخزّن تعادل أربعة أضعاف تلك الموجودة عند المرأة. ومن أجل دراسة هذه الفرضية، أجرينا دراسة تعتمد على فئران المختبر. تم اختيار مجموعتين تتكون كل واحدة من عشرة من فئران سبراغ دولي البالغة. تم قياس نسب "الفرتين" الأساسية في مصل الدم، وتم اعطاء الفئران واحدة من حميتين، حمية عادية أو حمية تحتوي على نسبة عالية من الحديد ولمدة أربعة أسابيع.

تم قياس نسب "الفرتين" في مصل الدم بعد أسبوعين أو بعد أربعة أسابيع. تم قتل خمسة فئران من كل مجموعة بعد أسبوعين وتم قتل الخمسة الأخرى من كل مجموعة بعد أربعة أسابيع. تم تجميع الدم لاستخدامه لقياس نسب الحديد و"الفرتين" بالإضافة إلى قياس نسب قوة الدم وعدد الخلايا ونسب الدهون المختلفة في أجسام هذه الفئران وتم كذلك دراسة الشرايين التاجية في هذه الفئران من ناحية تكوين تصلب الشرايين حيث تم دراسة هذه الشرايين نسيجياً على ثلاث مقاطع أُخذت لهذا الغرض.

وفي دراسة أخرى استعادية للرجال والنساء، تم أخذ النتائج والأرقام فيها من سجلات المستشفيات والمختبرات السريرية في المنطقة الشرقية للمملكة العربية السعودية، كان هنالك ارتباط ذو دلالة كبيرة وهام بين نسبة حدوث أمراض الشرايين التاجية وخصوصاً الجلطة القلبية والنسب العالية من "الفرتين" في مصل الدم.

الكلمات الدالة: استنفاذ الحديد، فئران، البشر، مخزون الحديد.