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Basal Endocrine Status in African Dietary Iron Overload

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Endocrine disturbances, notably diabetes, have been well described as a complication of iron overload due to hereditary hemochromatosis and β-thalassemia. Dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis has also been well documented. The pattern of iron loading in African iron overload with saturated transferrin is similar to that seen in hereditary hemochromatosis. In addition, many symptoms ascribed to pituitary dysfunction are common to both conditions. The present study was undertaken to assess whether a similar pattern of endocrine dysfunction occurs in African iron overload. Thirty subjects with African iron overload and transferrin saturation >50%, plus 30 age and sex matched normal controls were studied. An iron profile, fasting plasma glucose, cortisol, DHEA-S, LH, FSH, growth hormone, prolactin, TSH, and FT4 levels were measured in all 60 subjects as well as testosterone in the males and estradiol in the females. Iron loading in the subjects with increased transferrin saturation ranged from moderate to severe. No significant differences were found in the mean testosterone, estradiol, LH, DHEA-S, growth hormone, prolactin, or TSH levels between the subjects and normal controls. In female subjects, although within the normal range, the mean FSH level was significantly higher, probably due to their being somewhat older and in a more advanced stage of menopause than the control females. Mean cortisol concentrations were increased in both genders in the patient group, significantly so in the females; however, values were within the reference range. We conclude therefore that there appears to be no major impairment of endocrine function in the basal state in African iron overload subjects with moderate to severe degrees of iron loading.

Key Words: Endocrine status; iron overload; Africans.

Introduction

Iron overload, long considered a rarity, is now recognized as a common disorder of iron metabolism. It may occur as a genetic disorder, as in hereditary hemochromatosis and African iron overload, or as a secondary condition as in β-thalassemia. Endocrine disturbances, notably diabetes, have been well described as a complication of iron overload due to hereditary hemochromatosis and β-thalassemia. Dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis has also been well documented *(1,2)*. Impotence due to hypogonadal hypogonadism is frequently encountered as a presenting symptom in young subjects with hemochromatosis *(3)*, although no correlation between the degree of iron loading and sexual dysfunction has been demonstrated *(1)*. As far as we are aware, basal endocrine status has not been studied in subjects with African iron overload.

African iron overload was initially thought to develop as the result of a metabolic defect brought about by chronic malnutrition *(4)*. Subsequently, the dietary iron intake of affected rural African populations was shown to be very high, and there is now abundant evidence that the ingestion of large amounts of iron-rich traditional beer over many years is central to the development of this disease *(5)*. More recent studies suggest that a gene may be implicated in the pathogenesis of this condition *(6,7)*. It should be noted that the mutations of the *HFE* gene found in hereditary hemochromatosis are not found in subjects with African iron overload *(8)*.

The spectrum of iron loading in African dietary iron overload ranges from mild to severe. In individuals with a mild to moderate degree of excess hepatic iron, adverse consequences are not known to occur. However, in subjects with heavy iron loading, hepatic damage and portal cirrhosis occur, accompanied by saturated transferrin and iron accumulation in parenchymal cells of the pancreas, thyroid, adrenal glands, and heart. In this situation, the histological and clinical picture is very similar to symptomatic hereditary hemochromatosis *(9,10)*.

Although African iron overload is now less common in urban African populations *(11)*, it remains a public health issue in rural areas where more than 80% of the people of sub-Saharan Africa live *(12)*. The present study was under-

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Table 1
Biochemical Measurements of Subjects with African Iron Overload and Control Subjects

	Iron overloaded subjects	Control subjects
Number of subjects	30	30
Serum ferritin (μg/L)	1346 (583–3105)[a]	73 (25–214)
Transferrin saturation (%)	82 ± 17[b]	30 ± 10
Non-transferrin-bound iron (μm)	3.4 ± 1.9[b]	0

[a] Values are expressed as the geometric mean and SD range.
[b] Values are expressed as the mean ± SEM.

taken to investigate aspects of endocrine dysfunction in rural subjects with severe African iron overload.

Results

Biochemical measurements of iron status of the group chosen because of elevated transferrin saturation (the iron overloaded group) and control subjects are summarized in Table 1. The iron overloaded group had a mean transferrin saturation of 82% compared to a mean of 30% in the control group. The geometric mean ferritin level in the subjects with elevated transferrin saturations was 1346 compared to 73 μg/L in the control group. Among the 30 subjects, the serum ferritin concentration ranged from 366 to 18039 μg/L; 15 had serum ferritin concentrations <1000 μg/L and 9 had serum ferritin >2000 μg/L. Non-transferrin bound iron was present in the serum of all iron overloaded subjects and absent in all of the control group. There was no significant difference in the mean fasting glucose levels in the two groups (4.5 ± 0.6 vs 4.8 ± 0.3 mmol/L).

Demographic and hormonal data of the groups are summarized in Table 2. Female subjects, who were slightly, but not significantly, older than the female controls, had significantly higher mean FSH levels (58 ± 6 vs 34 ± 6 U/L; $p = 0.02$); however, all values were in the reference range (23–116 U/L). Analysis of the other pituitary hormones showed no significant differences between any of the groups and all values were in the reference ranges: LH males 1.5–9.3 IU/L, females 15.9–54.0 IU/L; growth hormone 0.2–13.0 mIU/L; prolactin males 2.1–17.7 μg/L, females 1.8–20.3 μg/L. Thyroid stimulating hormone (TSH) results were similar in all groups and fell within the reference range (TSH 0.3–5.5 mIU/L). Despite FT4 concentrations being within the reference range for all groups (10–20 pmol/L), the mean value for female subjects was significantly lower than female controls ($p = 0.02$).

Turning to the adrenal hormones, mean cortisol concentrations were higher in both male and female subjects compared with controls, significantly so in the female group (571 ± 47 vs 429 ± 45 nmol/L; $p = 0.04$), and when the genders were combined ($p = 0.04$). Mean cortisol values were within the reference range for morning samples (190–670 nmol/L). As a whole, there was no difference in mean DHEA-S levels between the genders or between the subject and control groups. However, about half of the study subjects were aged between 50 and 69 years (male subjects = 10, male controls = 8; female subjects = 5, female controls = 8). Analysis of this subgroup revealed that mean DHEA-S levels were similar in the male subjects and controls (3.8 ± 0.9 vs 2.7 ± 0.3 μmol/L), but in the female subjects, the level was significantly lower than the female controls (0.6 ± 0.1 vs 1.7 ± 0.3 μmol/L; $p = 0.04$). DHEA-S values were within the reference ranges for this age group: males 1.5–8.2 μmol/L, females 0.6–4.5 μmol/L. No significant differences were found in the mean testosterone levels of the males and estradiol levels of the females.

Table 2
Demographic and Hormonal Data of Subjects with African Dietary Iron Overload and Control Subjects[a]

	Iron overloaded subjects		Normal subjects	
	Males	Females	Males	Females
Number of subjects	15	15	15	15
Age (yr)	57 ± 4	60 ± 4	52 ± 3	53 ± 3
FSH (U/L)	9.7 ± 1.9	58 ± 6[b]	5.9 ± 0.8	34 ± 6
LH (IU/L)	5.8 ± 1.0	22 ± 1.8	4.6 ± 0.6	18 ± 3
Growth hormone (mIU/L)	3.5 ± 1.5	1.8 ± 0.6	2.8 ± 0.9	1.4 ± 0.3
Prolactin (μg/L)	8.1 ± 2.0	3.8 ± 0.5	8.0 ± 1.0	5.4 ± 0.7
TSH (mIU/L)	2.4 ± 1.0	1.8 ± 0.4	1.8 ± 0.2	3.1 ± 1.2
FT4 (pmol/L)	14 ± 0.5	12 ± 0.8[b]	14 ± 0.5	14 ± 0.8
Cortisol (nmol/L)	666 ± 57	571 ± 47[c]	541 ± 37	429 ± 45
DHEA-S (μmol/L)	3.8 ± 0.9	0.9 ± 0.2	3.6 ± 0.4	1.5 ± 0.4
Estradiol (pmol/L)	—	149 ± 21	—	100 ± 27[d]
Testosterone (nmol/L)	24 ± 2.6	—	28 ± 1.5	—

[a] Values are expressed as mean ± SEM.
[b] Significance of values in the patient group compared with normal subjects: $p = 0.02$.
[c] Significance of values in the patient group compared with normal subjects: $p = 0.04$.
[d] $n = 14$; one outlier >3670 excluded from data.

Discussion

Endocrine abnormalities may be found in patients with iron overload due to hereditary hemochromatosis and other iron loading diseases such as β-thalassemia. While diabetes is a frequent complication of hemochromatosis, the proportion of patients with symptoms of sexual dysfunction has been shown to be significantly greater than in a control group

matched for age, prevalence, and duration of diabetes *(2)*. The impaired sexual function of hemochromatosis patients has been related to hypogonadotrophic hypogonadism or testicular dysfunction and studies have shown that testosterone, FSH, and LH levels are significantly lower in hemochromatosis patients than in normal controls *(2,13,14)*.

Although the pattern of iron deposition in hereditary hemochromatosis and African iron overload differs, subjects with African iron overload, high transferrin saturations, and cirrhosis show evidence of parenchymal iron deposition very similar to that seen in hereditary hemochromatosis *(15)*. Recent work has shown that once the transferrin saturation exceeds 50%, non-transferrin-bound iron appears in the serum *(16)*. The presence of this abnormal iron fraction has been correlated with pathological changes in the liver *(17)* and would appear to be the major pathway leading to iron loading of parenchymal tissue, including the pituitary, and hepatic injury *(15,18)*. The serum ferritin level is an unreliable measurement of iron overload as it is influenced by inflammation and tissue damage *(15)*. While 50% of the subjects had a serum ferritin <1000 µg/L, they all had a high transferrin saturation and non-transferrin-bound iron in their serum. In addition, liver biopsies performed on seven of the subjects confirmed the presence of iron overload.

The present study has not shown any major abnormalities in the HPA axis in subjects with moderate to severe African iron overload. The higher mean FSH level in female subjects with iron overload is probably due to them being somewhat older, with a lower mean estradiol value indicating a more advanced stage of menopause, and cannot be ascribed to iron overload. A limitation of the study is that formal pituitary stimulation testing was not done. Although basal pituitary hormone levels were not different from those of the control subjects, it is possible that subtle degrees of pituitary dysfunction would have been demonstrated after hypothalamic releasing factor administration. Nevertheless, with pituitary dysfunction, the first hormones to be affected are usually the gonadotrophins (LH and FSH), and, in our study, the levels of these hormones were similar to, or elevated, in comparison with control subjects. However, this is an area that could be studied further.

Of interest is the finding that mean cortisol concentrations were higher in both male and female subjects compared with their normal counterparts. One explanation could be that this is the result of prolonged stress endured by such subjects, partly because of their increased susceptibility to recurrent infections *(19)*. Similar findings have been reported in patients suffering from other chronic illnesses *(20)*.

Approximately 90% of DHEA-S originates from the adrenal cortex and circulating concentrations reflect directly on adrenal androgen production *(21)*. There is, however, a gradual fall off in DHEA-S levels with advancing age. Although our 50–69 yr female subject group did have lower DHEA-S levels compared with the normal group, overall the values for both genders were within the physiological range. Whether or not DHEA-S replacement therapy in selected cases with very low levels might be of benefit remains to be determined.

In conclusion, there appears to be no major impairment of basal endocrine function in subjects with moderate to severe African dietary iron overload.

Materials and Methods

Subjects

This study was performed on a selection of subjects from a larger genetic study group comprising 180 rural-dwelling southern African subjects of predominantly Swazi and Shangaan extraction. Fasting blood samples were collected from all subjects between 06h00 and 08h00 on two successive days and a full medical history was taken from each person. Iron status was assigned on the basis of the mean values from both days. For the present study 30 iron overloaded subjects were selected from the above group on the basis of having a transferrin saturation >50% on successive days. All 30 subjects also had non-transferrin-bound iron, which is associated with parenchymal loading, present in their serum *(17)*. These subjects also had elevated serum ferritin concentrations. Iron overload was confirmed by liver biopsy in seven of these subjects, all of whom showed evidence of liver fibrosis. Thirty age and sex matched controls were selected from the same genetic study group on the basis of having a transferrin saturation <50%. Subjects and controls all came from the same ethnic background. Five of the male subjects and three of the males in the control group gave a history of impotence. There was no statistical difference between the testosterone levels in the group that reported impotence and the group that did not. None of the subjects were on any medication that might cause sexual dysfunction. In addition, subjects and controls were not taking adrenal corticosteroids or using any form of hormonal replacement therapy.

Study Design

Sera from subjects and controls were stored at –70°C until required. Informed consent was obtained from all subjects and the study was approved by the Committee for Research on Human Subjects (Medical) of the University of the Witwatersrand.

Measurement of Iron Status

Serum iron and iron binding capacity were performed according to the methods of the International Committee for Standardisation in Haematology *(22,23)*. An enzyme-linked immunosorbent assay was used for measuring serum ferritin *(24)*. The transferrin saturation was calculated by dividing the serum iron by the total iron binding capacity and multiplying by 100. Non-transferrin-bound iron was measured as previously described *(17)*.

Measurement of Glucose, Pituitary, Thyroid, Adrenal, and Gonadal Hormones

Fasting plasma glucose levels were measured by a standard oxidase method. Concentrations of the pituitary hormones, follicle stimulating hormone (FSH), luteinizing hormone (LH), and prolactin, as well as the thyroid hormones, thyroid stimulating hormone (TSH) and free thyroxine (FT4), were determined by direct chemiluminometric technology (ADVIA® Centaur™, Bayer Corporation, Diagnostics Division, East Walpole, MA). Sensitivities for these assays are as follows: FSH 0.3 U/L; LH 0.07 IU/L; prolactin 0.3 µg/L; TSH 0.01 mIU/L; FT4 1.3 pmol/L. Growth hormone levels were measured using an immunometric assay (Immulite®, Diagnostic Products Corporation, Los Angeles, CA), sensitivity 0.03 mIU/L. The adrenal hormones, cortisol and dehydroepiandrosterone-sulphate (DHEA-S), were measured by radioimmunoassay (ICN Biochemicals Inc., Costa Mesa, CA); sensitivity 3.0 nmol/L and 0.05 µmol/L, respectively. Levels of gonadal hormones, testosterone in males and estradiol in females, were measured using the chemiluminescent technology described above, sensitivity 0.35 nmol/L and 36.7 pmol/L, respectively. Interassay coefficients of variation were between 5% and 10% for all assays.

Statistical Analysis

Data were analyzed using the two-tailed Student's unpaired *t*-test (parametric) or the Wilcoxon signed rank test (nonparametric) as appropriate. A value of $p < 0.05$ was considered significant. Results are expressed as mean ± SEM or as the geometric mean and SD range.

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