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Body iron is a contributor to oxidative damage of DNA

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Abstract
The transition metal iron is catalytically highly active *in vitro*, and not surprisingly, body iron has been suggested to promote oxidative stress *in vivo*. In the current analysis we studied the association of serum ferritin concentration and serum soluble transferrin receptor concentration with daily urinary 8-hydroxydeoxyguanosine excretion, a marker of oxidative stress, in 48 mildly dyslipidemic men in East Finland. In multivariate linear regression analyses allowing for age, smoking, body mass index and physical exercise, serum ferritin concentration predicted the excretion rate at $B = 0.17$ (95% CI 0.08–0.26, $P = 0.001$), and serum soluble transferrin receptor to ferritin concentration ratio (TfR/ferritin) predicted the excretion rate at $B = -0.13$ (95% CI -0.21 to -0.05, $P = 0.002$). Our data suggest that body iron contributes to excess oxidative stress already at non-iron overload concentrations in these subjects.

Keywords: *Oxidative stress, iron, 8-hydroxydeoxyguanosine (8-OHdG), ferritin, transferrin receptor*

Introduction

The *in vitro* ability of iron and iron complexes to participate in free radical producing reactions has been demonstrated conclusively [1,2]. On the other hand, a number of physiological defences minimize the amounts of free iron in the body and there *is* substantially less evidence of iron contributing to harmful free radical reactions and oxidative stress *in vivo* [1].

One detectable consequence of free radical stress in the body is the oxidative modification of nucleic acids [3]. A DNA repair product, 8-OHdG, can be reliably measured from urine samples [4,5]. Excretion rate of 8-OHdG has been earlier associated at least with tobacco smoking, body mass index, extreme physical exercise and individual oxygen consumption [6–9].

Recently, Nakano and coworkers studied urinary 8-OHdG excretion in 2507 healthy Japanese and showed an age-dependent gender difference so that pre-menopausal females had lower and post-menopausal females higher 8-OHdG excretion rate as compared with males of the same age [10]. They also observed a statistically significant direct correlation between serum ferritin concentration and urinary 8-OHdG excretion rate [10].

Serum ferritin is a reasonably good indicator of body storage iron, but it does not reflect the functional iron pool as good [11]. For this purpose, especially at low body iron, serum soluble transferrin receptor (TfR) concentration is a usable indicator [12]. Combined, these two measurements construct an index that measures body iron stores from deficiency

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to overload [13,14]. For this reason, we assessed serum transferrin receptor in addition to serum ferritin, and studied the ratio of these with urinary excretion rate of 8-OHdG in mildly dyslipidemic healthy men in East Finland.

Materials and methods

Subjects

The randomized clinical trial "Antioxidant Supplementation in Atherosclerosis Prevention" (ASAP) study studied the effect of vitamins E and/or C supplementation on carotid atherosclerosis in 520 middle-aged men and postmenopausal women in East Finland [15]. All subjects had mild hypercholesterolemia at entry to the lead-in period, defined as serum cholesterol of 5.0 mmol/l (193 mg/dl) or more. The study consisted of 8-week dietary counselling with placebo lead-in phase and a 3-year double masked treatment period.

The present analysis is based on samples taken at the baseline visit of the last 48 men entered in the ASAP study during July–October, 1995. Subjects were instructed to abstain from eating for 12 h and from ingesting alcohol for 72 h before visit. 24-h urine sample was collected and after the subject had rested for 5 min in a sitting position, venous blood sample was drawn with Venoject vacuum tubes (Terumo, Osaka, Japan). No tourniquet was used.

The ASAP study was approved by the joint Research Ethics Committee of the University of Kuopio and the Kuopio University Hospital. All Subjects gave their written informed consent to participate in the study.

Measurements

Urinary excretion of 8-OHdG was measured from 24-h sample by automated three dimensional high performance liquid chromatography with electrochemical detection. The method has been described in detail previously [6]. Measured concentration was adjusted for total volume collected to get an estimate of the total daily excretion. Serum ferritin concentration was measured with an immunoradiometric assay (BioRad IRMA, Hercules, CA), based on a double antibody technique. Serum TfR concentration was measured using the IDeA Transferrin Receptor Immunoenzymometric Assay (ORION Diagnostica, Turku, Finland). The TfR/ferritin ratio was computed as serum TfR concentration (μg/l) divided by serum ferritin concentration (μg/l).

Body mass index (BMI) was computed as the ratio of weight in kilograms divided by height in meters squared. Number of conditioning leisure time physical activity sessions per week and number of cigarettes smoked daily were assessed with a self-administered questionnaire [15].

Statistical methods

All computations were performed with SPSS 13.0 for Windows statistical software. Non-parametric crude associations were studied using Spearman rank-order correlation coefficients. Multivariate adjusted linear regression models were fitted to allow for potential confounder variables age (years), smoking (yes–no), BMI (kg/m^2) and conditioning leisure time physical activity (conditioning is defined as physical activity that causes at least shortness of breath or sweating, or both) (sessions per week). In the latter analyses logarithmic transformations were used to normalize the skewed distributions. In addition, difference in daily urinary 8-OHdG excretion between high (>200 μg/l) vs. lower serum ferritin concentration, as well as high (>3000 ng/l) vs. lower serum transferrin receptor concentration were tested with non-parametric Mann–Whitney test. A two sided $P < 0.05$ was taken as statistically significant.

Results

Baseline characteristics of the study subjects are given in Table I. Spearman correlation coefficients between urinary 8-OHdG (nmol/d) and serum ferritin (μg/l), serum TfR (ng/l), and TfR/ferritin (μg/l/μg/l), were 0.358 ($P = 0.014$), -0.039 ($P = 0.790$), and -0.279 ($P = 0.055$), respectively. Regression coefficient for serum ferritin concentration in predicting daily urinary 8-OHdG excretion, in a multivariate model adjusting for age, smoking, BMI and conditioning

Table I. Baseline characteristics of the 48 male study subjects.

Variable	Mean ± SD	Minimum	Maximum
Urinary excretion of 8-hydroxy-deoxyguanosine (nmol/d)	32.7 ± 9.9	15.8	58.8
Serum ferritin concentration (μg/l)	129 ± 177	11	1240
Serum transferring receptor concentration (mg/l)	2.60 ± 1.01	1.28	6.95
Serum TfR/ferritin concentration ratio (μg/l/μg/l)	63.6 ± 111	1.9	632
Age (years)	57.0 ± 7.2	45.4	68.9
Smoking (cigarettes/day)	7.1 ± 10.3	0	40
Body mass index (kg/m^2)	26.1 ± 2.8	19.1	32.5
Conditioning leisure time physical activity (sessions/week)	3.4 ± 3.8	0	16

Table II. Association between logarithm of fasting serum TfR/ferritin ratio and logarithm of daily urinary 8-OHdG excretion (nmol/d) in 48 middle aged healthy men. A multivariate adjusted linear regression model.

Variable	Regression coefficient	Standardized regression coefficient	95% CI, low	95% CI, high	*P* two sided
Log serum TfR/ferritin	−0.129	−0.461	−0.206	−0.051	0.002
Age (years)	0.005	0.107	−0.008	0.017	0.470
Smoking status (yes vs. no)	0.086	0.140	−0.096	0.276	0.346
Body mass index (kg/m^2)	0.141	0.200	−0.010	0.054	0.176
Conditioning leisure time physical activity (sessions/week)	0.002	0.027	−0.021	0.026	0.853

leisure time physical activity, was 0.165 (95% CI 0.076 to 0.255, $P = 0.001$). This model explained 20.6% ($P = 0.012$) of the total variation in daily 8-OHdG excretion. In a respective multivariate model with serum TfR/ferritin the regression coefficient was −0.129 (95% CI −0.206 to −0.051, $P = 0.002$) (Table II). This model explained 16.5% ($P = 0.028$) of the total variation in daily 8-OHdG excretion. Serum TfR concentration did not predict daily urinary 8-OHdG excretion in multivariate adjusted models. Age, smoking status, BMI, or leisure time exercise did not predict statistically significantly urinary 8-OHdG in these models.

Scatter plot of urinary 8-OHdG by TfR/ferritin is presented in Figure 1.

To study the extremes of body iron distribution, we tested low functional iron (high serum TfR concentration) and high stored iron (high serum ferritin concentration) with 8-OHdG excretion rate. In these analyses, high serum TfR concentration was associated with low excretion rate (mean rank 13.9 vs. 26.6, $P = 0.017$), while high serum ferritin concentration was not as clear predictor of high daily 8-OHdG excretion (mean rank 30.7 vs. 23.1, $P = 0.149$).

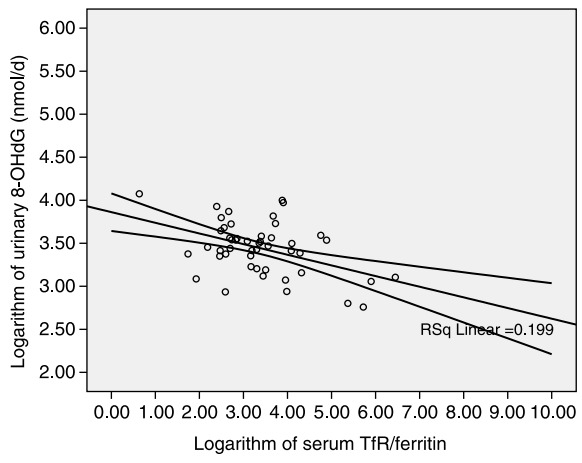

Figure 1. Fitted linear regression line with 95%CI between serum TfR/ferritin ratio and daily urinary 8-OHdG excretion.

Discussion

In the present study, both serum ferritin concentration and serum TfR/ferritin ratio were associated with daily urinary 8-OHdG excretion, a marker of oxidative damage *in vivo*.

Both DNA damage rate and ferritin biosynthesis may be increased during inflammation. However, our subjects were generally healthy, and neither blood leukocyte count nor plasma fibrinogen concentration was associated with serum ferritin and TfR/ferritin concentrations (data not shown). Furthermore, exclusion of a lone high ferritin value did not change the results (data not shown). Smoking, gender, BMI, extreme physical exercise and oxygen expenditure have been identified earlier as determinants of urinary 8-OHdG excretion rate [6–9]. Adjustment for these in our analyses did not markedly affect the observed associations. However, the relatively low number of subjects limited us from performing extensive analyses of further confounders.

Increased circulating soluble TfR concentration reflects cellular serum transferrin receptor synthesis, which is up-regulated in shortage of functional iron. On the basis of literature, the iron depleted state—no stored iron in the presence of normal haemoglobin—might be optimal with regard to iron related pathophysiology [16]. In keeping with this, our data with high sTfR concentration associating with low 8-OHdG excretion rate suggest that low iron stores may be protective against excess oxidative stress.

On the other hand, serum ferritin, a marker of high body iron stores, was associated with higher rate of 8-OHdG excretion over the whole range, suggesting no particular treshold effect.

Experimental evidence on iron and 8-OHdG favour iron acting a contributor to oxidative DNA damage. Studies by Borollo and coworkers from Italy, in a rodent colitis model, show both dietary iron deprivation and pharmacological iron chelation to decrease DNA damage rate [17,18]. Yoshiji and coworkers found iron deficiency to inhibit development of pre-neoplastic lesions and production of 8-OHdG in rat livers [19]. Kato and coworkers found combined phlebotomy and dietary iron restriction to decrease hepatic 8-OHdG

formation and to protect against hepatocellular carcinoma in chronic hepatitis C patients [20]. A study by Holmberg and coworkers studied 8-OHdG excretion in hereditary hemochromatosis subjects, not supporting an association. However, in this study the subjects had been under treatment before the study and had only marginally elevated stored iron at the time of the study [21]. Instead, in a study by Kom and coworkers, increased urinary excretion of 8-iso-prostaglandin -another marker of oxidative stress—was observed in hereditary hemochromatosis subjects. Furthermore, this increased excretion was reversable by phlebotomy, giving further support for body iron as the major determinant [22].

The only other study relating body iron with oxidative DNA damage in healthy subjects was conducted in 2507 men and women in Tokyo, Japan [10]. In that study, Nakano and coworkers observed almost no change by age in urinary 8-OHdG excretion in males, but did observe an age-dependent increase in the excretion rate in women, most marked at the time of menopause. Also, serum ferritin concentration had a statistically significant direct correlation with urinary 8-OHdG excretion rate in both genders. Together these observations suggested that menstrual blood loss which keeps iron stores low protects young females against excess oxidative DNA damage. However, only crude associations were reported and no multivariate models were fitted [10].

In our study, we extended iron assessment from serum ferritin concentration to serum TfR concentration and the ratio of these, and fitted multivariate models to control over potential confounders. In conclusion, our data give support to the previous suggestion that body iron promotes oxidative stress, and extend that low body iron could be particularly protective. Whether the observed associations are due to stored, rather inactive, iron or due to the so called labile iron pool (LIP) iron, may be debated. It is not clear where LIP iron comes from in the healthy cell but it seems that it reflects the size of total body iron pool. Recently, Gackowski and coworkers observed a direct correlation between lymphocyte native 8-OHdG concentration and amount of LIP [23], and LIP may well prove to be a key player in iron-promoted oxidative stress, including oxidative DNA damage [24,25].

Taken together, our data suggest that body iron, even within the normal reference range, is pro-oxidative *in vivo*, and that keeping body stored iron as low as possible, might benefit health.

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