Three decades of gastroenterology in Soweto South Africa: from descriptive to scientific observations

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Chapter 13

Iron, Ascorbate and Copper Status of Sowetan Blacks with Calcific Chronic Pancreatitis

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Summary
Vitamin C can be used to overcome oxidative stress and ease pain in chronic pancreatitis. But its use is deprecated in conditions of tissue iron overload, because its bioactive form, ascorbate, can accelerate free-radical reactions that are driven by transition metals. We measured iron, ascorbate and copper in Sowetan Blacks (RSA) with chronic pancreatitis, obtaining serum/plasma from 14 consecutive patients and 15 controls. Compared with data from corresponding groups in Manchester, African samples had less ascorbate (p<0.0001), but more caeruloplasmin (p<0.0001). African and British controls had comparable iron and iron-binding capacity. Plasma from African patients had less ascorbate than that from African controls (p<0.005) and in six samples, ferritin exceeded 300 µg/l (677 pmol/l). Low-molecular-mass iron or copper, capable of participating in free radical reactions, was not detected. British patients, had similar caeruloplasmin levels to African patients but higher ascorbate levels. There is no evidence of iron overload in our African samples. Outwardly healthy controls from Soweto have elevated levels of caeruloplasmin, possibly to compensate for dietary deficiency of ascorbate. Persistent oxidative stress is a unifying feature of chronic pancreatitis, but its degree is higher in African than British patients. Supplements of vitamin C should be safe in Blacks of southern Africa.

Introduction
In short-term\(^ 1\) and long-term\(^ 2\) studies at Manchester, UK, supplements of micronutrient and antioxidants helped to overcome oxidative stress and concurrently eased both background pain and the tendency to recurrent attacks in patients with chronic pancreatitis. By varying the antioxidant prescription in three placebo-controlled trials, the combination of vitamin C and methionine was identified as the key to this therapeutic success.\(^ 4\) Admission statistics from Baragwanath Hospital at Soweto, on the outskirts of Johannesburg, RSA, suggest that chronic pancreatitis is currently endemic in this township (I. Segal, unpublished observations): extensive pancreatic calculi and crippling abdominal pain are common features at presentation, and the disease runs an aggressive course towards premature death. The disease emerged here in the 1970s, about a decade after repeal of the legislation which formerly forbade the sale of western-type liquor to African Blacks.\(^ 6\) A more recent surge has coincided with industrialization and urbanization, resulting in large scale migration of rural Blacks to the city.\(^ 7\) Formerly, southern African Blacks ('Bantus') were known to the medical literature from descriptions of 'Bantu siderosis'.\(^ 8-11\) 'Bantu' men brewed acidic beer in
iron pots such that the leached iron resulted in a dietary excess of 100 mg of iron (1.8 mmol) per day. As time went by, the excess iron was deposited in the liver, reticuloendothelial tissues, endocrine and exocrine pancreas and the eventual development of hepatic cirrhosis came to be equated with iron toxicity: although glucose intolerance was reported, chronic pancreatitis was not. Iron toxicity was also held responsible for the heightened oxidation of ascorbate, precipitating scurvy and osteoporosis, because vitamin C was already in short supply through poor diets. Paradoxically, repletion with the vitamin was accompanied by a rise in serum iron level, which raised suspicion that the vitamin may influence the pattern of tissue iron deposition and release. This fear was strengthened when myocardial depression was reported after administration of vitamin C to thalassaemic patients with transfusional iron-overload, another condition in which ascorbate deficiency and poor bone healing were recognized. Thereafter clinicians were exhorted not to prescribe vitamin C in states of iron overload.

This advice posed a dilemma for us when recent collaborative studies of patients with calcific chronic pancreatitis (CCP) at Soweto showed profound depletion of ascorbate along with toxic metabolite stress. These studies also showed that outwardly healthy Sowetans were in a state of subclinical oxidative stress, linked with poor intake of vitamin C. The potential scope of vitamin C supplementation for both prophylaxis and treatment made it necessary to reinvestigate iron status in Blacks of southern Africa, in the light of what is now known about the tissue toxicity of iron and how ascorbate and copper interact.

Methods

African patients

Consecutive African patients with calcific chronic pancreatitis (CCP) (n=14) and outwardly healthy hospital workers (n = 15) were investigated at Soweto over a four-week period in 1993. They had previously co-operated in studies of oxidative stress and related aspects. The mean age of the patients was 40 years, range 27-52; 13 were male and one female, and their mean body mass index was 20, range 15-27. Alcoholism was the dominant aetiological factor. Background abdominal pain was the dominant clinical problem, and two patients had recovered from an attack of pancreatitis 3 weeks earlier. Exocrine pancreatic function was markedly impaired in four patients, moderately impaired in six and well preserved in the others. Two patients had overt liver disease with biochemical profiles indicating cholangiohepatitis. Insulin-requiring diabetes had developed in one patient.
The control group consisted of 13 men and two women, mean age 34 years, range 19-47, and mean body mass index 23, range 19-31. The absence of past or present medical symptoms was the only criterion for acceptance in the study. Social histories showed that nine men drank alcohol daily, but in only one of them was this clearly excessive, > 120 g for the previous 10 years.

**British patients**

We have previously reported plasma ascorbate and vitamin C levels in Manchester patients. For the present report, this information has been modified by including only patients with pancreatic calculi, CCP. There were very few of these in our 1981 report on increased copper oxidase activity. We therefore analysed stored samples of serum/plasma from 15 patients with CCP for transition metal status. Pancreatic secretory function was usually well preserved in this group which included 12 men and three women, of mean age 43 years, range 24-68, and mean body mass index 21, range 16-28. Alcoholism, defined as consumption of at least 80 g daily, was an aetiological factor in 12 patients; heavy cigarette usage, at least 20 per day, may have contributed to the disease in this subgroup and a further two patients; long-term treatment with anticonvulsants may have been relevant in one patient and daily close exposure to occupational hydrocarbons in 10 patients. There was no clinical evidence of liver disease in the group, but routine liver function profiles showed an increase in γ-glutamyltranspeptidase activity in five patients. Diabetes had developed in six patients and required treatment by insulin in four of them.

For the studies of transition metal status, 18 outwardly healthy male hospital workers donated blood samples. Their mean age was 40 years, range 22-63 years, and all of them drank alcohol on a social basis.

**Sampling, processing and analysis**

For the African studies, venous blood was obtained after an overnight fast, processed for measurement of antioxidants and free-radical oxidation products in serum and/or plasma and then stored in suitable fractions at -70°C. They were despatched in dry ice to Manchester at weekly intervals. The transit time was always < 16 h so that samples were still deep-frozen on arrival. They were analysed within the established stability periods for each assay. Plasma vitamin C, which includes dehydroascorbic and diketogulonic acids, was measured by a colorimetric method with lower detection limit of 1.7 μmol/l (equivalent to 0.3 mg/l) and
Ascorbic acid was measured by HPLC using a method with lower detection limit of 2.8 μmol/l (0.5 mg/l)². Routine haematology and liver function profiles were obtained through the Johannesburg hospital service.

Commercial kits were used for serum iron, iron binding capacity (colorimetric method based on ferrozine, Boehringer-Mannheim), transferrin, caeruloplasmin (immunonephelometric techniques, Behring) and ferritin (ELISA). Copper was measured by atomic absorption spectroscopy. Using molecular masses of 135 000 for caeruloplasmin, 76 500 for transferrin and 443 000 for ferritin, the results for these proteins were converted to SI units. Low molecular-mass iron and copper were measured at London, using methods that have been described in detail previously.³²,³³

Statistical analysis

Because of the small numbers in each group, results were compared by Student's t test, with ANOVA and correction for multiple comparison, and also the non-parametric Mann-Whitney U test. Correlations were examined by parametric or non-parametric methods as appropriate. Ferritin levels were interpreted by reference to a published nomogram.³⁰ Differences were regarded as significant at p<0.05 (two-tailed tests).

Results

Ascorbate

Ascorbate results have been published previously¹⁵ but for the present paper the information on Manchester patients has been restricted to those with pancreatic calculi. Plasma from African controls had less ascorbate than that from British controls (p<0.0001). Patients with CCP in each area had lower ascorbate than their corresponding regional controls (p<0.0001 in Manchester, p<0.005 in Johannesburg), but African patients had substantially lower ascorbate than did British patients (p<0.005) (Figure 1, Table 1). By reference to vitamin C levels in the same samples, it was evident that ascorbate reflected vitamin C availability in African controls, British controls and British patients, but was excessively oxidised in African patients (data not shown).¹⁵,¹⁷
<table>
<thead>
<tr>
<th>Measurements</th>
<th>Controls</th>
<th>Patient</th>
<th>Significance of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Manchester</td>
<td>Jo'burg</td>
<td>Manchester</td>
</tr>
<tr>
<td>Ascorbate (µmol/l)</td>
<td>60.5 ± 4.63</td>
<td>20.4 ± 4.77</td>
<td>12.1 ± 2.14</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0001</td>
<td>p&lt;0.005</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Iron (µmol/l)</td>
<td>16.4 ± 1.06</td>
<td>14.9 ± 1.56</td>
<td>14.8 ± 2.71</td>
</tr>
<tr>
<td>Iron binding capacity (µmol/l)</td>
<td>51.7 ± 1.26</td>
<td>55.6 ± 1.96</td>
<td>54.3 ± 2.67</td>
</tr>
<tr>
<td>Iron-saturation (%)</td>
<td>31.6 ± 1.78</td>
<td>27.4 ± 2.97</td>
<td>27.0 ± 4.30</td>
</tr>
<tr>
<td>Transferrin (µmol/l)</td>
<td>40.0 ± 1.70</td>
<td>42.0 ± 1.57</td>
<td>35.7 ± 2.36</td>
</tr>
<tr>
<td>Ferritin (pmol/l)</td>
<td>112 (26-492)</td>
<td>189 (18-1963)</td>
<td>174 (9.48-3548)</td>
</tr>
<tr>
<td>Copper (µmol/l)</td>
<td>15.3 ± 0.50</td>
<td>18.7 ± 1.73</td>
<td>20.3 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caeruloplasmin (µmol/l)</td>
<td>2.03 ± 0.07</td>
<td>2.96 ± 0.21</td>
<td>3.07 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0001</td>
<td></td>
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</tr>
</tbody>
</table>

Data are mean ±SE except for ferritin where the geometric mean and standard range is given. *Comparison by Student's t test (two-tailed), after logarithmic transformation of data on ferritin for which the comparisons were restricted to males since reference ranges are different for males and females. The application of non-parametric tests yielded a similar outcome except in the case of ferritin, where use of the Mann-Whitney U test indicated that African patients had higher levels than local controls (p<0.05) and that African controls and patients had higher values than their British counterparts.
Iron

Serum iron and iron-binding capacity were similar in Johannesburg and Manchester groups (Table 1). Transferrin accounted for the spare iron-binding capacity, which lay between 50 and 95% in the African samples (r=0.9899, p<0.0001 in controls; r=0.9294, p<0.0001 in patients).

Hospital reference ranges for ferritin were different for males and females, as has been observed previously.\textsuperscript{20} The levels were compared only among males, who constituted the majority in each subgroup. There were no differences by Students t test. However, the Mann-Whitney U test showed that African patients had higher levels than local controls (p < 0.05) and that African controls and patients had higher values than their British counterparts (p<0.05 for each comparison). In some 30% of patients at each centre, ferritin values were in the upper quartile of the reference range or higher (Figure 1) despite the ample iron-binding capacity in those samples.

The published nomogram\textsuperscript{20} suggested a combination of liver disease and inflammation as the explanation for the high ferritin levels in these samples (Figure 2). African and British samples with ferritin >300 μg/l (677 pmol/l), the 95% upper confidence limit from the large-scale study in the USA,\textsuperscript{20} were analysed for low-molecular-mass iron by the bleomycin assay.\textsuperscript{32} Bleomycin cannot remove iron which is correctly loaded onto carrier proteins or that which is structurally associated with haem-proteins\textsuperscript{32}: a modification, the iron-binding antioxidant assay, is based on the ability of biological samples to inhibit or promote damage to DNA by iron-bleomycin complexes. There was no free iron in any sample. The iron-binding antioxidant assay gave a mean (±SE) of 29±4.4% inhibition compared with 28±1.4% for healthy controls in the UK.

Copper

There was a good correlation between serum copper and caeruloplasmin concentrations in African and British samples (r=0.8912, p<0.0001; r=0.8491, p<0.0001, respectively). For copper, the only significant difference was among the British subgroups, where patients had higher values than controls (p < 0.001, Table 1). However, in the case of caeruloplasmin, African controls had higher levels than British controls (Figure 1): the difference was ironed out upon development of CCP, when patients from each area had higher values than their respective reference ranges (Table 1). There was an inverse correlation between caeruloplasmin and ascorbate in the African samples as a whole (Figure 3) but not when subgroups were considered separately (r= -0.3873 controls, -0.1983 patients). The specialized
assay for low-molecular-mass copper was applied to the seven African samples with high ferritin levels: in three of these samples, caeruloplasmin concentration exceeded the reference range. There was no low-molecular-mass copper in any sample. The same was true for Manchester samples with ferritin and/or caeruloplasmin concentrations above the reference ranges.

Iron, copper and ascorbate in chronic pancreatitis

Figure 1: Composite showing distribution of data on ascorbic acid, available iron-binding capacity, ferritin and caeruloplasmin in controls (blocks) and patients at Manchester (open triangles) and J’burg (filled triangles). All comparisons were by student’s test.
Discussion

It is now known that the superoxide anion free radical (O$_2^-$), hydrogen peroxide (H2O2) and lipid peroxides (LOOH) are purposefully produced to fulfil physiological functions. The low reactivity of these species ensures that cellular organelles are not damaged in the process. However, in the presence of low-molecular-mass iron (Fe $^{2+}$) or copper (Cu $^+$) complexes, the potentially devastating hydroxyl radical (OH.) is quickly formed.24,32,34 Efficient proteins bind, store and oxidize these transition metals and thus control their intracellular and extracellular pools. Such proteins are regarded as primary antioxidants, their function buttressed by secondary antioxidants of which the bulk derives from the diet; ascorbate, α-tocopherol and glutathione, from sulphur amino acids, are the most important. They act synergistically and display metabolic redundancy.35

We have reported free radical marker,15 drug metabolism17 and micronutrient antioxidant status18 of Sowetan African patients and controls who participated in the present investigation of iron and copper metabolism. Those results reinforced the concept that toxic metabolite stress, involving induced pancreatic cytochromes P450, is a unifying feature in the geographic pathology of CCP and causally connected with the disease.36 The results also showed that outwardly healthy Sowetans are in a state of subclinical oxidative stress.15 The main objective of the present study was to establish whether iron overload could be held responsible for pancreatic damage and also subclinical liver damage in these people. Between the 1920s and 1960s when descriptions of 'Bantu siderosis' appeared, little was known of how excess iron might damage cells and there were no laboratory tests to indicate that this threat was real in certain states of iron-overload. In the past two decades, this situation has changed. The ferroxidase I activity of the copper-containing protein caeruloplasmin is now accepted as being pivotal to its antioxidant function.37 If mishandled or stored, it degrades to form oxidized lipid-copperprotein complexes (ferroxidase II) which, unlike ferroxidase I, are not inhibited by azide.37,38 It has recently been shown that ferritin, too, is a ferroxidase and cytoprotectant.25 The synthesis of iron-binding and iron-oxidizing proteins is stepped-up to counter any burst of free radical activity. Should they fall short, even transiently as in patients with the adult respiratory distress syndrome,39 Fe$^{2+}$ and Cu$^{+}$ could quickly wreak damage.
Under these circumstances, and these alone, ascorbate can switch from antioxidant to pro-oxidant by holding transition metals in their catalytically active reduced forms.

\[ \text{Fe}^{2+} + \text{Cu}^{2+} \text{ Ferroxdase I} \rightarrow \text{Fe}^{3+} + \text{Cu}^{+} \text{ Ferroxdase I} \]

\[ \text{O}_2 + 4\text{H}^{+} + 4\text{Cu}^{+} \text{ Ferroxdase I} \rightarrow 2\text{H}_2\text{O} + 4\text{Cu}^{2+} \text{ Ferroxdase I} \]

\[ \text{Fe}^{3+} + \text{ Ascorbate} \rightarrow \text{Fe}^{2+} + \text{Dehydroascorbate} \]

\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH} + \text{OH} \]

Against this background, the results of our study indicate that: (i) iron-overload cannot be blamed for heightened free radical activity and pancreatic damage among Sowetan Africans today; (ii) high serum ferritin is a non-specific phenomenon;\(^{20}\) (iii) outwardly healthy Sowetans are indeed in a state of subclinical oxidative stress;\(^{17}\) (iv) this problem is amplified upon development of CCP; (v) persistent oxidative stress is a unifying feature in the geography of CCP, but its degree is higher in African than in British patients.

The first finding could perhaps have been anticipated from previous reports in Sowetan 'Bantus' which highlighted the divergent time-trends, in the 15-20 year period from 1960, between decreasing tissue iron accumulation but increasing frequency of hepatic cirrhosis\(^{40}\) with emergence of CCP.\(^{7}\) A study of patients with CCP in the early 1980s concluded that a minority had iron-overload\(^{7}\) and that elevated serum ferritin in several of the other patients was a non-specific manifestation\(^{20}\) of tissue injury (I. Segal and A.P. MacPhail, unpublished). The Western pattern of liver and pancreatic disease was ascribed to the higher consumption by these people of commercial liquor with alcohol content of some 14%, and less intake of home-brewed liquor which contained only 3% alcohol but was rich in iron.\(^{7,40}\)

Provided that the information on the group of 29 African patients in our investigation is representative of the community at large, then the results indicate that iron overload is not prevalent among Sowetan Africans in the 1990s. Thus, serum retained ample capacity to bind and oxidize iron, such that there was no free iron detected by the specialized tests even when serum ferritin levels were very high (Figure 1, Figure 2). The non-specificity of elevated serum ferritin was recognized some time ago\(^{20}\) Among the possible explanations for the high values noted in several samples in the present investigation, subclinical oxidant-related damage to the liver\(^{55}\) and kidney\(^{41}\) has been reported in European patients, as was first noted in an autopsy report of CCP some 200 years ago.\(^{42}\) The outcome in regard to iron sequestering proteins contrasts sharply with that in idiopathic and transfusional haemochromatosis, when iron transporters are saturated, the bleomycin assay detects low-molecular-mass iron, and the
iron binding antioxidant assay shows stimulation instead of the normal inhibition. This type of contemporaneous information has not hitherto been available, but it is noteworthy that in some 30% of individuals with 'Bantu siderosis' and liver iron content >2%, there was said to be little by way of histological damage to the liver and also that serum contained ample capacity to bind iron because the production of transferrin was apparently stepped-up.

Figure 2: Relationship between total iron-binding capacity and ferritin in serum samples from African controls (filled circles), African patients with CCP (filled triangles) and three samples with ferritin > 300 ug/ml from Manchester patients with CCP (open triangles). The nomogram is adapted from the work of Lipschitz et al.
These observations support the notion that iron of itself is a weak fibrogenic agent, but potentiates the detrimental effect of other exogenous toxins and/or nutrient deficiencies. Clearly, iron toxicity cannot be blamed for subclinical oxidative stress in the Sowetan community nowadays, as indicated by elevated caeruloplasmin (Figure 1) and lipid peroxide levels in outwardly healthy individuals. The best explanation is that inaffordability of foods rich in vitamin C leaves tissues vulnerable to the normal quota of reactive oxygen species, despite a compensatory increase in caeruloplasmin production. In this context, a study from the UK showed an inverse correlation between plasma ascorbate and caeruloplasmin in babies but not in adults. Our findings (Figure 3) suggest that under conditions where ascorbate deficiency is sustained, the inverse relationship between these primary and secondary antioxidants is maintained into adulthood. The increment in serum caeruloplasmin upon development of CCP results in levels of the protein that are as high as in adults with the respiratory distress syndrome, a condition which is linked with oxidative stress and to which patients with pancreatitis are prone. Although caerulopiasmin was measured by an immunonephelometric method in the present study, we have previously reported increased copper oxidase activity in British patients with chronic pancreatitis; it has recently been confirmed that under normal circumstances a linear correlation exists between caeruloplasmin concentration and copper oxidase, ferroxidase I, activity. It may be that there is a ceiling above which caeruloplasmin levels cannot rise even when oxidative stress persists, and under these circumstances ascorbate is left vulnerable to oxidation, whether by an excess of reactive oxygen species or by reactive metabolites that are inadvertently produced with xenobiotics are processed by cytochromes P450. We conclude that there should be no hazard from the use of vitamin C among Sowetan Africans, whether to assess its value as prophylaxis against chronic pancreatitis or to facilitate treatment of painful disease. Indeed, we recently reported the safe use of high doses of ascorbate intravenously, in conjunction with other antioxidants, in a young British woman who was emaciated from crippling pain due to CCP and turned out to have idiopathic haemochromatosis.

Acknowledgments

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Schofield for help in collating the results; Carroline Thorpe, Victoria Powell and Rachel Candolker for excellent secretarial assistance; and Sandra Roe for the Figures.

![Graph](image-url)

**Figure 3: Relationship between concentration of ascorbic acid and caeruloplasmin in material from African controls (circles) and patients with CCP (triangles).**

**References**


33. Gutteridge JMC. Copper-phenanthroline induced site specific oxygen radical damage to DNA. Detection of loosely-bound trace copper in biological fluids. Biochem J 1984; 218:983-5.


