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Workshop Report

Risk of iron overload in carriers of genetic mutations associated with hereditary haemochromatosis: UK Food Standards Agency workshop

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The UK Food Standards Agency convened a group of expert scientists to review current research investigating diet and carriers of genetic mutations associated with hereditary haemochromatosis. The workshop concluded that individuals who are heterozygous for the C282Y mutation of the *HFE* gene do not appear to respond abnormally to dietary Fe and therefore do not need to change their diet to prevent accumulation of body Fe.

Iron overload: Hereditary haemochromatosis: Food Standards Agency workshops

Background

The UK Food Standards Agency (FSA) convened a workshop on 12 November 2003 on the nutritional implications of mutations of the gene linked to haemochromatosis. The results from recently completed studies were presented, both FSA- and non-FSA funded, and the workshop was chaired by Professor Jim Cook.

Introduction

Hereditary haemochromatosis is a common, autosomally recessive, genetic disorder of Fe metabolism. It is characterised by excessive Fe absorption, resulting in the accumulation of high levels of Fe body stores. If left untreated it can have serious consequences, including diabetes, arthritis and cirrhosis of the liver (Bothwell & MacPhail, 1998).

Mutation of the *HFE* gene, which is involved in Fe absorption and storage, has been identified as the major cause of haemochromatosis (Feder *et al.* 1996). Two mutations of the *HFE* gene, C282Y and H63D, have been linked with the disease. In the UK, most patients with haemochromatosis are homozygous for the C282Y mutation of the *HFE* gene (The UK Haemochromatosis Consortium, 1997), although the majority of homozygotes in the population do not develop clinical symptoms leading to a diagnosis of haemochromatosis (McCune *et al.* 2002). In a minority of cases, there may be mutations in the genes encoding transferrin receptor 2, ferroportin-1, haemojuvelin and hepcidin (Roetto *et al.* 2003; Swinkels *et al.* 2006).

Mutations in the *HFE* gene are common in populations of European origin (Merryweather-Clarke *et al.* 2000). In a study of blood donors in the UK, 15 % were heterozygous, and 0·7 % homozygous, for C282Y, while 25 % were heterozygous, and 2 % homozygous, for the H63D mutation (Jackson *et al.* 2001). Fe stores (as measured by transferrin saturation (TS), serum ferritin (sFn) concentration and serum Fe binding capacity) were observed to increase in the order: wild types, H63D heterozygotes, C282Y heterozygotes, H63D homozygotes, compound heterozygotes and C282Y homozygotes; although sFn concentrations were no higher in H63D heterozygotes and C282Y heterozygous women than in wild types. If these increases in TS and sFn truly reflect increases in tissue Fe levels, subjects carrying *HFE* mutations may be at increased risk of disease.

Abbreviations: FSA, Food Standards Agency; sFn, serum ferritin; TfR, serum transferrin receptor; TS, transferrin saturation.

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Assessment of body tissue iron

Professor Jim Cook described a technique for measuring body tissue Fe, either the surplus of storage Fe or the deficit of tissue Fe, which uses the serum transferrin receptor (TfR):sFn ratio (Cook *et al.* 2003). A low TfR:sFn ratio indicates high Fe stores. The method was demonstrated to estimate the body Fe content of healthy subjects (Cook *et al.* 2003). The method provides individual values for storage Fe and also permits a substantial reduction in the number of subjects needed to assess response to an intervention study. The use of this method to compare different studies will not be possible, however, until the assay has been standardised.

The hepatic protein, hepcidin, found in urine and blood, is thought to regulate dietary Fe uptake and its distribution in the body by controlling the concentration of ferroportin, an Fe exporter present on the surface of absorptive enterocytes, macrophages and hepatocytes (Nemeth *et al.* 2004). Hepcidin might serve as a useful indicator of body tissue Fe in future studies (Kemna *et al.* 2005).

Genetic influence on iron status: morbidity and mortality

Professor Mark Worwood presented epidemiological evidence of morbidity and mortality outcomes associated with hereditary haemochromatosis. A study in the USA observed no increase in the frequency of the most common symptoms of haemochromatosis in subjects homozygous for C282Y, or heterozygotes for both H63D and C282Y, relative to wild-type subjects (Beutler *et al.* 2002). The frequency was only higher for C282Y homozygotes reporting liver problems or with an increased aspartate transaminase activity. A study in Wales of haemochromatosis patients observed that only 1·2 % of adults homozygous for C282Y had been diagnosed with Fe overload (McCune *et al.* 2002); furthermore, the frequency of homozygosity for C282Y is not under-represented in old age (Willis *et al.* 1999; Coppin *et al.* 2003).

Several prospective studies have suggested that heterozygotes for C282Y may be at increased risk of CVD (Roest *et al.* 1999; Tuomainen *et al.* 1999; Rasmussen *et al.* 2001); however, other prospective studies have observed no association between *HFE* mutations and CVD risk (Gunn *et al.* 2004; Ellervik *et al.* 2005).

The iron hypothesis of atherosclerosis

Professor Joe Marx presented evidence on the hypothesis that body Fe stores are associated with CVD risk (Sullivan, 1981). Several prospective studies have observed an association between increased sFn concentrations and increased carotid atherosclerosis (Kiechl *et al.* 1997; Wolff *et al.* 2004) and CVD (Salonen *et al.* 1992; van der A *et al.* 2005). Ferritin is an acute-phase protein, which is increased in inflammation. Chronic inflammation (for example, as assessed by plasma C-reactive protein concentrations) is associated with an increased risk of CVD (Danesh *et al.* 1998). One study, which adjusted for C-reactive protein concentrations, found an association between sFn concentrations and ischaemic stroke risk in postmenopausal women (van der A *et al.* 2005). Other prospective studies, however, have found little or no evidence that sFn concentrations are a risk factor for CVD (Danesh & Appleby, 1999; Knuiman *et al.* 2003).

TS has not been associated with CVD risk (Sempos *et al.* 1994; Reunanen *et al.* 1995; Danesh & Appleby, 1999; van der A *et al.* 2005), although the TfR:sFn ratio was associated with increased CVD risk in one study (Tuomainen *et al.* 1998). The results from epidemiological studies, therefore, remain equivocal.

In plasma, Fe is normally bound to transferrin, making it less reactive. If Fe is in a labile and reactive form in plasma, unbound to transferrin, it can react with cells and molecules. Non-transferrin-bound Fe has been found in the plasma of patients with Fe-overload conditions (Grootveld *et al.* 1989), homozygotes and heterozygotes for C282Y (de Valk *et al.* 2000) and in patients with end-stage renal disease (Kooistra *et al.* 2002). Non-transferrin-bound Fe has also been shown to increase the expression of adhesion molecules in cultured endothelial cells and monocytes, representing an early phase of atherosclerosis (Kartikasari *et al.* 2004). These studies provide some support for the Fe hypothesis; however, a recent prospective study found no association between CVD risk and plasma non-transferrin-bound Fe concentrations in postmenopausal women (van der A *et al.* 2006).

The effects of diet on iron absorption and iron stores in *HFE* genotypes

Regulation of Fe absorption is affected by mutations in the *HFE* gene. The consequences for C282Y heterozygotes, however, are not clear. One study suggested that absorption from diets of high bioavailability may be increased in heterozygotes (Lynch *et al.* 1989), but these findings were not replicated in another study (Hunt & Zeng, 2004).

Professor Sue Fairweather-Tait presented results from an FSA-funded project that measured Fe absorption from a diet containing highly bioavailable Fe (high meat, fish and poultry, high vitamin C, low phytate) extrinsically labelled with ^{57}Fe-enriched ferrous sulfate and a diet of fortified cereal products extrinsically labelled with ^{54}Fe-enriched ferrous sulfate, over a 5 d period in C282Y heterozygotes (*n* 15), C282Y/H63D compound heterozygotes (*n* 5), and wild-type men (*n* 15). An erythrocyte incorporation technique was used to evaluate the effects of high dietary Fe on Fe homeostasis. Overall, the differences in Fe absorption between the various genotypes were small and genotype did not affect Fe absorption (Roe *et al.* 2005). There was no association between genotype and sFn concentrations.

The relationship between habitual diet and body Fe stores (Hb, sFn, TS, TfR) in C282Y heterozygote (*n* 46) and wild-type (*n* 92) men aged 40 years and over was also assessed, to determine the extent to which diet modulates Fe accumulation. A positive association was found between haem-Fe intake and sFn concentration. The C282Y heterozygotes had slightly higher TS, but genotype only accounted for 5 % of the variation in TS and there was no difference in other measures of Fe stores.

Professor Janet Cade presented results from another FSA-funded project where 2531 women, from the UK Women's Cohort Study (aged 35–69 years at baseline), were genotyped for C282Y and H63D mutations and assessed for Fe intake and stores. Although C282Y homozygotes (*n* 31) had higher

sFn concentrations, there was no difference between heterozygotes for C282Y or H63D (*n* 726) and wild types (*n* 1774). Heterozygotes for C282Y did have slightly higher TS concentrations than wild-type subjects (Greenwood *et al.* 2005).

Dietary assessment showed that sFn concentration was positively associated with haem-Fe intake. Women heterozygous for the C282Y mutation responded to haem Fe similarly to normal women with regard to their sFn concentrations. A strong interaction between genotype, haem-Fe intake and raised sFn concentrations was observed, however, primarily in postmenopausal women who were homozygous for C282Y (Cade *et al.* 2005).

Overall, these studies confirm previously published reports that C282Y heterozygotes have a slightly higher TS compared with wild-type controls, but do not have a higher sFn concentration (Jackson *et al.* 2001; Beutler *et al.* 2002). Absorption of Fe, and Fe stores, do not appear to be significantly higher in C282Y heterozygotes than in wild types.

Discussion

Individuals who are heterozygous for the C282Y mutation of the *HFE* gene appear to respond normally to dietary Fe and do not need to alter their diet to avoid increased levels of stored Fe.

The following research recommendations were identified:

(1) Standardisation of the serum TfR assay to encourage its use as a marker of body Fe content, in conjunction with sFn, in clinical and population studies;
(2) Adjustment for inflammation and infection in studies associating chronic disease with sFn concentrations;
(3) Prospective studies to explore the possible link between penetrance of *HFE* mutations in relation to dietary patterns and other factors associated with Fe metabolism and risk of chronic disease.

Participants

Professor James Cook, University of Kansas Medical Center, USA; Professor Joe J. M. Marx, University Medical Centre, Utrecht, The Netherlands; Professor Mark Worwood, University of Wales College of Medicine; Professor Sue Fairweather-Tait, Institute of Food Research; Dr Janet Cade, Leeds University; Dr Mark Roe, Institute of Food Research; Dr Birgit Teucher, Institute of Food Research; Dr Tim Key, Scientific Advisory Committee on Nutrition (SACN); Professor Peter Aggett, SACN; Dr Kathryn Robson, John Radcliffe Hospital, Oxford; Dr Alison Merryweather Clark, John Radcliffe Hospital, Oxford; Dr Hélène Coppin, INSERM U563, CHU Purpan, Toulouse; Dr Marie Paule Roth, INSERM U563, CHU Purpan, Toulouse; Dr Gavin Willis, Norfolk & Norwich University; Dr Jennifer Moreton, Leeds University; Dr Anne McCune, Gloucestershire Royal Hospital; Dr Margaret Ashwell, FSA Programme Adviser; Dr Alison Tedstone, FSA; Rachel Elsom, FSA; Mamta Singh, FSA; Cheryl White, FSA.

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