

Hemochromatosis

ABSTRACTS

- Bloem MW., 1990. Vitamin A intervention: short-term effects of a single, oral, massive dose on iron metabolism.
- Borch-lohnsen B., 1997. [Primary hemochromatosis and dietary iron]
- Brown KE., 1997. Effect of vitamin E supplementation on hepatic fibrogenesis in chronic dietary iron overload.
- Guyader D., 1998. Noninvasive prediction of fibrosis in C282Y homozygous hemochromatosis.
- Hallberg L., 1998. Does calcium interfere with iron absorption?
- Kaltwasser JP., 1998. Clinical trial on the effect of regular tea drinking on iron accumulation in genetic haemochromatosis.
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- Olsson KS., 1997. The effect of withdrawal of food iron fortification in Sweden as studied with phlebotomy in subjects with genetic hemochromatosis.
- Piperno A., 1998. Classification and diagnosis of iron overload.
- Piperno A., 1998. Heterogeneity of hemochromatosis in Italy.
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- Stal P., 1998. Defective iron metabolism in genetic hemochromatosis. The mechanisms remain unknown in spite of genetic advances.
- Shaheen NJ., 1998. Clinical characteristics of hereditary hemochromatosis patients who lack the C282Y mutation.
- Worwood M., 1998. Haemochromatosis.
- Ramrakhiani S., 1998. Hemochromatosis: advances in molecular genetics and clinical diagnosis.
- Stal P., 1998. [Defective iron metabolism in genetic hemochromatosis. The mechanisms remain unknown in spite of genetic advances]
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Vitamin A intervention: short-term effects of a single, oral, massive dose on iron metabolism.

Bloem MW, Wedel M, van Agtmaal EJ, Speek AJ, Saowakontha S, Schreurs WH. Department of Clinical Biochemistry, TNO-CIVO Toxicology and Nutrition Institute, Zeist, The Netherlands.

Am J Clin Nutr 1990 Jan;51(1):76-9

A group of 134 school children aged 3-9 y, with signs of conjunctival xerosis, from the rural area of the Sakorn Nakhon province in Northeast Thailand were selected for a controlled study on the short-term effect (2 wk) of a single, oral high dose of vitamin A on iron metabolism. After collection of the baseline data, children within villages were randomly assigned to receive the capsules (n = 65) or serve as control subjects (n = 69). Two weeks after supplementation significant increases of retinol, retinol-binding protein,

hemoglobin, hematocrit, serum iron, and saturation of transferrin were found in the supplemented group. Ferritin concentrations did not change significantly. These short-term changes completely exclude seasonal effects and change in morbidity. This study provides further evidence of a causal association between vitamin A and iron metabolism. In areas where vitamin A deficiency is endemic, periodic massive vitamin A dose programs can also improve iron status of the population.

[Primary hemochromatosis and dietary iron] (in Norwegian).

Borch-Iohnsen B Ernaeringsinstituttet Universitetet i Oslo.

Tidsskr Nor Laegeforen (Norway) Oct 10 1997, 117 (24) p3506-7

Primary haemochromatosis is characterized by an unusually high degree of iron absorption resulting in the accumulation of excessive amounts of tissue iron. Excess stores of iron are removed by repeated phlebotomy. Health personnel and a number of patients with primary haemochromatosis have expressed their desire for advice on special diets to try and reduce the number of phlebotomies per year. This article gives advice on how patients with primary haemochromatosis can decrease their dietary iron intake and how they can put together meals to obtain low bioavailability, and therefore a lower iron absorption. The diet should be varied and be rich in bread and cereals, and fruit and vegetables. The amount of meat, Norwegian brown whey cheese (iron supplemented) and alcohol should be limited. Tea or coffee with meals will reduce iron absorption. Food rich in ascorbic acid (fruit and fruit juice) should be avoided with meals. Ascorbic acid supplements are not recommended.

Effect of vitamin E supplementation on hepatic fibrogenesis in chronic dietary iron overload

Brown K.E.; Poulos J.E.; Li L.; Soweid A.M.; Ramm G.A.; O'Neill R.; Britton R.S.; Bacon B.R. B.R. Bacon, Div. of Gastroenterology/Hepatology, Dept. of Internal Medicine, Saint Louis Univ. Hlth. Sci. Center, 3635 Vista Ave., St. Louis, MO 63110-0250 USA

American Journal of Physiology - Gastrointestinal and Liver Physiology (USA), 1997, 272/1 35-1 (G116-G123)

It has been suggested that lipid peroxidation plays an important role in hepatic fibrogenesis resulting from chronic iron overload. Vitamin E is an important lipid-soluble antioxidant that has been shown to be decreased in patients with hereditary hemochromatosis and in experimental iron overload. The aim of this study was to determine the effects of vitamin E supplementation on hepatic lipid peroxidation and fibrogenesis in an animal model of chronic iron overload. Rats were fed the following diets for 4, 8, or 14 mo: standard laboratory diet (control), diet with supplemental vitamin E (200 IU/kg, control + E), diet with carbonyl iron (Fe), and diet with carbonyl iron supplemented with vitamin E (200 IU/kg, Fe + E). Iron loading resulted in significant decreases in hepatic and plasma vitamin E levels at all time points, which were overcome by vitamin E supplementation. Thiobarbituric acid-reactive substances (an index of lipid peroxidation) were increased three- to fivefold in the iron-loaded livers; supplementation with vitamin E reduced these levels by at least 50% at all time points. Hepatic hydroxyproline levels were increased twofold by iron loading. Vitamin E did not affect hydroxyproline content at 4 or 8 mo but caused an 18% reduction at 14 mo in iron-loaded livers. At 8 and 14 mo, vitamin E decreased the number of alpha-smooth muscle actin-positive stellate cells in iron-loaded livers. These results demonstrate a dissociation between lipid peroxidation and collagen production and suggest that the profibrogenic action of iron in this model is mediated through effects which cannot be completely suppressed by vitamin E.

Noninvasive prediction of fibrosis in C282Y homozygous hemochromatosis.

Guyader D; Jacquelinet C; Moirand R; Turlin B; Mendler MH; Chaperon J; David V; Brissot P; Adams P; Deugnier Y Clinique des Maladies du Foie and INSERM Unite 49, Rennes, France. Dominique.Guyader@univ-rennes1.fr

Gastroenterology (UNITED STATES) Oct 1998, 115 (4) p929-36

BACKGROUND & AIMS: The diagnosis of hemochromatosis is now possible for C282Y homozygous patients using noninvasive molecular genetic tests. The aim of this study was to define noninvasive factors predictive of severe fibrosis (bridging fibrosis or cirrhosis) to avoid unnecessary liver biopsies in such patients. **METHODS:** Clinical and biological data were recorded at the time of diagnosis in 197 French C282Y homozygous patients, 52 (26%) of whom had severe fibrosis. Variables significantly linked to severe fibrosis using univariate analysis were entered into a multivariate stepwise analysis. These variables were combined to obtain a simple index allowing for prediction of severe fibrosis. **RESULTS:** Serum ferritin, hepatomegaly, and serum aspartate aminotransferase were selected using multivariate analysis. Their combination applied to the 96 patients with ferritin level of ≤ 1000 microgram/L, normal aspartate aminotransferase values, and absence of hepatomegaly showed that no severe fibrosis was encountered in this subgroup of patients. The results were validated in 113 C282Y homozygous patients in Canada with a good reproducibility of negative prediction but a poor reproducibility of the positive prediction of severe fibrosis. **CONCLUSIONS:** In C282Y homozygous patients, the diagnosis of severe fibrosis relies on liver biopsy, but absence of severe fibrosis can be accurately predicted in most patients on the basis of simple clinical and biochemical variables.

Does calcium interfere with iron absorption?

Hallberg L

Am J Clin Nutr 1998 Jul;68(1):3-4

No abstract.

Clinical trial on the effect of regular tea drinking on iron accumulation in genetic haemochromatosis

Kaltwasser J.P.; Werner E.; Schalk K.; Hansen C.; Gottschalk R.; Seidl C. J.P. Kaltwasser, Medizinische Klinik III, Zentrum der Inneren Medizin, Johann Wolfgang Goethe-Universität, Theodor-Stern-Kai 7, D-60596 Frankfurt am Main Germany

Gut (United Kingdom) , 1998, 43/5 (699-704)

Background - Black tea is known to be a potent inhibitor of intestinal absorption of non-haem iron at least in healthy subjects. **Aims** - To investigate this effect in patients with genetic haemochromatosis, and, more importantly, the effect of regular tea drinking on the accumulation of storage iron in these patients over one year. **Patients** - Investigations were carried out on 18 patients with clinically proven genetic haemochromatosis. For the study of storage iron accumulation, they were separated into a group instructed to drink a particularly tannin rich tea regularly with meals and a control group. **Methods** - Intestinal iron absorption from a test meal was measured using whole body counting. Body iron stores were evaluated quantitatively by exhaustive phlebotomy, using haemoglobin, saturation of serum iron binding capacity, and serum ferritin for the assessment of body iron status. **Results** - A significant reduction in iron absorption was observed when the test meal was accompanied by drinks of tea instead of water. In the tea drinking group, the increase in storage iron was reduced by about one third compared with that of the control group. **Conclusions** - Regular tea drinking with meals reduces the frequency of phlebotomies required in the management of patients with haemochromatosis.

Antioxidants for hemochromatosis.

Last, W.

Int. Clin. Nutr. Rev. 1991; 11(2): 71-4.

No abstract available.

The effect of withdrawal of food iron fortification in Sweden as studied with phlebotomy in subjects with genetic hemochromatosis.

Olsson KS; Vaisanen M; Konar J; Bruce A Department of Medicine, Molndal Hospital, Sweden.

Eur J Clin Nutr (England) Nov 1997, 51 (11) p782-6

OBJECTIVES: The iron fortification of food in Sweden, the highest in the world, was withdrawn 1st January 1995, because the effect upon target groups was considered to be uncertain. We wanted to study the effect of such a dietary experiment.

DESIGN: Comparative cross over study.

SETTING: Out patient service and Blood Bank.

SUBJECTS: Sixteen men aged 24-73 y on maintenance phlebotomy after treatment for iron overload. One was excluded because of inflammatory disease.

INTERVENTIONS: Quantitative phlebotomy with serial measurements of Hb conc., % transferrin saturation and serum ferritin concentration.

MAIN OUTCOME MEASURES: Iron absorption was measured by phlebotomy during two periods, with and without iron fortification. 1 g Hb = 3.4 mg Fe.

RESULTS: Iron absorption was significantly reduced ($P < 0.001$) when iron fortification was withdrawn from a mean of 4.27 ± 1.2 to 3.63 ± 1.1 mg/d. The difference of 0.65 mg/d (95% c.i.0.32-0.97) corresponds to the fraction of iron derived from fortification.

Intervals between donations had to be extended from 59 +/- 15 to 69 +/- 17 d ($P < 0.01$) to avoid induction of iron deficiency anemia. The iron content of the fortified diet averaged 15.4 mg/d, of which the fortified fraction constituted 4.1 mg/d (27%). The relative bioavailability of carbonyl iron used as fortificant was 38%.

CONCLUSIONS: The relative bioavailability of carbonyl iron used as fortificant was higher than previously reported. Target groups such as menstruating females will probably be affected by a higher prevalence of iron deficiency when food is no longer fortified. People with genetic hemochromatosis will accelerate into clinical disease at a slower rate.

Classification and diagnosis of iron overload.

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Haematologica (Italy) May 1998, 83 (5) p447-55, 078

BACKGROUND AND OBJECTIVE: Iron overload is the result of many disorders and could lead to the development of organ damage and increased mortality. The recent description of new conditions associated with iron overload and the identification of the genetic defect of hereditary hemochromatosis prompted us to review this subject and to redefine the diagnostic criteria of iron overload disorders.

EVIDENCE AND INFORMATION SOURCES: The material examined in the present review includes articles published in the Journals covered by the Science Citation Index and Medline. The author has been working in the field of iron overload diseases for several years and has contributed ten of the papers cited in the references.

STATE OF THE ART AND PERSPECTIVES: Iron overload can be classified on the basis of different criteria: route of access of iron within the organism, predominant tissue site of iron accumulation and cause of the overload. Excess iron can gain access by the enteral route, the parenteral route, and placental route during fetal life. The different distribution of iron within parenchymal or reticuloendothelial storage areas indicates different pathogenetic mechanisms of iron accumulation and has relevant implications in terms of organ damage and prognosis of the patients. Iron overload may be either primary, resulting from a deregulation of intestinal iron absorption as in hemochromatosis or secondary to other congenital or acquired conditions. Diagnosis of iron overload can be suspected on the basis of clinical data, high transferrin saturation and/or serum ferritin values. However, several hyperferritinemic conditions are not related to iron overload, but may imply severe disorders (inflammations, neoplasia) or a deregulation of ferritin synthesis (hereditary hyperferritinemia-cataract syndrome), and iron overload secondary to aceruloplasminemia, and the recently described dysmetabolic-associated liver iron overload syndrome, are characterized by low or normal transferrin saturation levels. Liver biopsy is still very useful in the diagnostic approach to iron overload disorders, by defining the amount and the distribution of iron within the liver. The analysis of HFE gene mutations (C282Y and H63D) is a simple and strong tool in the diagnostic work out of iron overload conditions. (60 Refs.)

Heterogeneity of hemochromatosis in Italy.

Piperno A; Sampietro M; Pietrangelo A; Arosio C; Lupica L; Montosi G; Vergani A; Fraquelli M; Girelli D; Pasquero P; Roetto A; Gasparini P; Fargion S; Conte D; Camaschella C Istituto di Scienze Biomediche, Università di Milano, Divisione di Medicina I, Ospedale San Gerardo, Monza, Italy.

Gastroenterology (United States) May 1998, 114 (5) p996-1002

BACKGROUND & AIMS: Patients with hemochromatosis show variable phenotype expression. We evaluated the frequency of hemochromatosis gene (HFE) mutations and the contribution of HFE genotype, ancestral haplotype, ethnic background, and additional factors (alcohol intake, hepatitis viruses, and beta-thalassemia trait) to the severity of iron overload in a large series of Italian patients with a hemochromatosis phenotype.

METHODS: HFE genotype was studied in 188 patients. Phenotype evaluation was available in 153 men and 20 women and was based mainly on iron removed. HFE genotype was determined by a polymerase chain reaction restriction assay and ancestral haplotype through D6S265 and D6S105 microsatellite analysis.

RESULTS: The frequency of C282Y homozygotes was 64%, with a decreasing gradient from north to south. C282Y homozygotes showed more severe iron overload than the other HFE genotypes. In the same group, ancestral haplotype was associated with a more severe phenotype. Additional factors may favor the development of a relatively mild hemochromatosis phenotype in patients nonhomozygous for the C282Y mutation.

CONCLUSIONS: Hemochromatosis in Italy is a nonhomogenous disorder in which genetic and acquired factors are involved. In patients with a single or no HFE mutation, further studies will enable a differentiation between true genetic disorders and

interactions between genetic and acquired factors.

Hemochromatosis: advances in molecular genetics and clinical diagnosis.

Ramrakhiani S; Bacon BR Department of Internal Medicine, Saint Louis University School of Medicine, MO 63110-0250, USA.

J Clin Gastroenterol (UNITED STATES) Jul 1998, 27 (1) p41-6

Hereditary hemochromatosis (HH) is a human leukocyte antigen-linked inherited disease that is characterized by inappropriately high absorption of iron by the gastrointestinal mucosa. The spectrum of disease presentation is changing with more and more patients now being identified before they are symptomatic with complications of iron overload. A candidate gene for HH, called HFE, was identified in 1996, and a test for the gene is commercially available. A review of the recent identification of the gene and its implications for clinical diagnosis and therapy is presented. We also propose an algorithm for evaluation of patients for HH. Early diagnosis and appropriate therapy can prevent significant morbidity and mortality associated with the development of end-organ complications of HH. The understanding of the C282Y and H63D mutations is still evolving, and the algorithm and the contribution of various heterozygous mutations to the diagnosis and management of iron overload need to be confirmed by further clinical and genetic studies. (30 Refs.)

Understanding iron absorption and metabolism, aided by studies of hemochromatosis.

Roeckel IE; Dickson LG Central Kentucky Blood Center, Lexington 40504, USA.

Ann Clin Lab Sci (UNITED STATES) Jan-Feb 1998, 28 (1) p30-3

Duodenal iron absorption from food is selectively blocked to prevent iron intoxication. The prime example of pathologic increase in intestinal iron absorption is seen in patients with hemochromatosis. They suffer iron damage to the heart, liver, and other tissues resulting in premature death if the iron is not removed by vigorous phlebotomy. Examples of overcoming the intestinal barrier to iron are alcohol consumption, vitamin preparations with vitamin C, and iron consumed by individuals without anemia. Endogenous generation of excess iron by hemolysis, owing to abnormal hemoglobin or many transfusions, are not controlled by the intestinal barrier. (24 Refs.)

SUGGESTED READING

Hereditary haemochromatosis mutation frequencies in the general population.

Bradley LA; Johnson DD; Palomaki GE; Haddow JE; Robertson NH; Ferrie RM Foundation for Blood Research, Scarborough, Maine 04070-0190, USA.

J Med Screen (England) 1998, 5 (1) p34-6

OBJECTIVES: This study aims to expand our knowledge of the general population frequency of two mutations, C282Y and H63D, identified in the candidate gene for hereditary haemochromatosis, and to determine whether the testing can be performed using routinely obtained cheek-brush (buccal) samples.

SETTING: Banked buccal lysate samples, randomised and coded for anonymity, from a cohort of couples who underwent prenatal cystic fibrosis screening in Maine.

METHODS: A multiplex ARMS test was performed on buccal cell lysates to identify the two mutations.

RESULTS: Genotype frequencies found among the 1001 subjects studied (502 women, 499 men) were: seven C282Y homozygotes, 22 C282Y/H63D compound heterozygotes, 97 C282Y heterozygotes, 17 H63D homozygotes, 246 H63D heterozygotes, and 612 individuals with no detectable mutation. The allele frequencies for C282Y and H63D were 0.066 and 0.151, respectively.

CONCLUSIONS: Observed genotype frequencies in Maine are consistent with expectations and with consensus data from five smaller studies. Combined mutational analysis data indicate that homozygosity for C282Y (the genotype found in about 85% of subjects with diagnosed hereditary haemochromatosis) occurs in 51 per 10,000 white subjects of northern European heritage; the corresponding total hereditary haemochromatosis prevalence of about 60 per 10,000 is consistent with previous estimates. The study also confirms that H63D would not be useful in general population screening for hereditary haemochromatosis.

Factors affecting the rate of iron mobilization during venesection therapy for genetic hemochromatosis.

Adams PC London Health Sciences Centre, University of Western Ontario, Canada.

Am J Hematol (United States) May 1998, 58 (1) p16-9

Although progressive iron accumulation is a characteristic feature of genetic hemochromatosis, the factors affecting the rate of iron mobilization by venesection have not been established. Venesection records were analyzed in 77 hemochromatosis homozygotes to study the factors affecting the rate of iron mobilization by venesection. The rate of iron mobilization was the iron removed divided by the time required to deplete iron stores (serum ferritin < 50 microg/L). Mean duration of venesection therapy was 1.4 years (range 0.44-3.6 years). All patients completed the therapy and there were no significant adverse effects. Rate of iron mobilization was higher in cirrhotics compared to non-cirrhotic patients ($P = 0.04$). Iron mobilization was inversely related to intestinal radioiron absorption ($r = -0.45$, $P = .01$). There was no significant relationship between iron mobilization and patient age, gender, serum ferritin, and hepatic iron concentration. Iron mobilization is increased in cirrhotics and patients with lower intestinal iron absorption. Venesection therapy is safe and well tolerated in all age groups.

Hemochromatosis and iron needs.

Halliday JW Queensland Institute of Medical Research, Bancroft Centre, Royal Brisbane Hospital, Queensland, Australia.

Nutr Rev (United States) Feb 1998, 56 (2 Pt 2) ps30-7; discussion s54-75

Although iron is an essential dietary requirement, the amount absorbed by the body is well regulated and depends on body iron stores and on dietary iron availability. There is very little iron excreted under normal conditions. Iron deficiency is a worldwide problem but iron overload, as seen in the inherited disease, hemochromatosis, is a major cause of morbidity in some Caucasian populations. This is a problem particularly where there is an adequate dietary iron intake and especially in males. A mutation has recently been described in an MHC Class I-like gene (HFE) that encodes for a protein (HFE) of 343 amino acids. The molecule contains a signal sequence peptide-binding region, alpha, and alpha(2) domains, and an immunoglobulinlike alpha(3) domain, in addition to a transmembrane region and a small cytoplasmic tail. It is a candidate gene for hemochromatosis. Several possibilities as to the function of this gene and the corresponding protein have been suggested but none has yet been confirmed. The mutation has been detected by several different groups in 80%-100% of subjects with the disease. However, in one study, 18%-20% of patients with the mutation did not exhibit significant iron overload. The discovery of this gene has important implications for both clinical studies and the elucidation of the pathways of iron metabolism. (41 Refs.)

Clinical trial on the effect of regular tea drinking on iron accumulation in genetic haemochromatosis

Kaltwasser J.P.; Werner E.; Schalk K.; Hansen C.; Gottschalk R.; Seidl C. J.P. Kaltwasser, Medizinische Klinik III, Zentrum der Inneren Medizin, Johann Wolfgang Goethe-Universitat, Theodor-Stern-Kai 7, D-60596 Frankfurt am Main Germany

Gut (United Kingdom), 1998, 43/5 (699-704)

Background - Black tea is known to be a potent inhibitor of intestinal absorption of non-haem iron at least in healthy subjects.

Aims - To investigate this effect in patients with genetic haemochromatosis, and, more importantly, the effect of regular tea drinking on the accumulation of storage iron in these patients over one year. **Patients** - Investigations were carried out on 18 patients with clinically proven genetic haemochromatosis. For the study of storage iron accumulation, they were separated into a group instructed to drink a particularly tannin rich tea regularly with meals and a control group.

Methods - Intestinal iron absorption from a test meal was measured using whole body counting. Body iron stores were evaluated quantitatively by exhaustive phlebotomy, using haemoglobin, saturation of serum iron binding capacity, and serum ferritin for the assessment of body iron status.

Results - A significant reduction in iron absorption was observed when the test meal was accompanied by drinks of tea instead of water. In the tea drinking group, the increase in storage iron was reduced by about one third compared with that of the control group.

Conclusions - Regular tea drinking with meals reduces the frequency of phlebotomies required in the management of patients with haemochromatosis.

Defective iron metabolism in genetic hemochromatosis. The mechanisms remain unknown in spite of genetic advances]

Stal P; Hagen K; Hultcrantz R Gastroenterologiskt Centrum, Huddinge Sjukhus.

Genetic haemochromatosis (GH) is one of the most common hereditary diseases, with a prevalence of 1-5/1000 in the Western world. In 90 per cent of cases a mutation is found in an MHC-class-like gene designated HFE, involving a substitution at position 282 of the HFE protein and resulting in defective binding of beta(2)-microglobulin. Animals with beta(2)-microglobulin deficiency develop iron overload, indicating this protein to be involved in the regulation of iron metabolism. Hepatic iron overload results in increased production of oxygen free radicals and peroxidation of membrane lipids, thus causing damage to lysosomes, mitochondria and the endoplasmic reticulum. These cellular events may progress to cell death, fibrogenesis, and the development of liver cirrhosis which is associated with a 200-fold increase in risk of hepatocellular carcinoma. In addition to the risk of diabetes, arthralgia, cardiac arrhythmia, pituitary insufficiency and hypogonadism, iron excess is also associated with aggravation of the cytotoxic effects exerted on hepatocytes by other agents such as alcohol or hepatotropic viruses. The treatment of iron overload in GH consists of weekly venesection until the serum ferritin level is normalized, followed by maintenance therapy. Survival rates are normal if the disease is detected and treated before complications have developed. (45 Refs.)

Clinical characteristics of hereditary hemochromatosis patients who lack the C282Y mutation. Shaheen NJ; Bacon BR; Grimm IS Division of Digestive Diseases and Nutrition, University of North Carolina, Chapel Hill 27599-7080, USA.

Hepatology (UNITED STATES) Aug 1998, 28 (2) p526-9

Approximately 85% of patients with typical hereditary hemochromatosis (HH) are homozygous for the C282Y mutation (C282Y/C282Y) in the recently identified candidate gene for HH. However, some HH patients are instead homozygous for the wild-type allele (wt/wt) at this locus. These wt/wt patients may represent a phenotypically similar, but genotypically different, heritable trait, or may be unrecognized cases of secondary iron overload. The purpose of this study is to provide an in-depth analysis of the wt/wt HH patients identified in the original description of the HH gene, and to compare them with 62 patients from the same analysis who were homozygous for the C282Y mutation. Eighteen of the 21 wt/wt HH patients from the original study were assessed for 14 historical and laboratory variables, including previously unrecognized causes of secondary iron overload, the heritability of iron overload and liver disease, and other clinical characteristics. Ten of these 18 wt/wt HH patients (55.6%) were found to have previously unrecognized causes for secondary iron overload compared with 3 of 62 (4.8%) of the C282Y/C282Y patients ($P < .001$). The remaining 8 wt/wt patients had no recognizable etiology of secondary iron overload. None of the 18 wt/wt patients had a family history of iron overload or liver disease, compared with 58% of the C282Y/C282Y patients ($P < .001$). When compared with C282Y homozygotes, the 8 wt/wt patients without secondary iron overload had a higher presenting hepatic iron index (HII) (9.5 vs. 4.7; $P = .01$). We conclude that, in this series of patients, over half of the wt/wt HH patients possessed previously unrecognized causes of secondary iron overload, and therefore, may have been misdiagnoses. If these cases are excluded, the number of false-negative tests is decreased, and the sensitivity of the mutational analysis is increased. However, there is a subgroup of wt/wt patients who have typical hemochromatosis without an identifiable cause of secondary iron overload. These patients may have more severe iron loading than C282Y homozygotes. (21 Refs.)

Haemochromatosis.

Worwood M Department of Haematology, University of Wales College of Medicine, Cardiff, UK.

Clin Lab Haematol (ENGLAND) Apr 1998, 20 (2) p65-75

Genetic haemochromatosis (GH) is the most common, autosomal recessive disorder in Northern Europe. The studies which led to the identification of the HFE gene are described. In the UK over 90% of patients with GH are homozygous for the C282Y mutation of this gene. This mutation is confined to populations of European origin. The significance of another mutation, H63D, in causing iron overload is less certain. Preliminary studies on the localization of the protein and the effects of the mutations are described. Genetic testing and the measurement of iron status now provide the means to allow for widespread testing for the prevention of iron overload and its consequences. However, questions remain about the clinical penetrance of GH. (81 Refs.)

Hemochromatosis: advances in molecular genetics and clinical diagnosis.

Ramrakhiani S; Bacon BR Department of Internal Medicine, Saint Louis University School of Medicine, MO 63110-0250, USA.

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implications for clinical diagnosis and therapy is presented. We also propose an algorithm for evaluation of patients for HH. Early diagnosis and appropriate therapy can prevent significant morbidity and mortality associated with the development of end-organ complications of HH. The understanding of the C282Y and H63D mutations is still evolving, and the algorithm and the contribution of various heterozygous mutations to the diagnosis and management of iron overload need to be confirmed by further clinical and genetic studies. (30 Refs.)

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Stal P; Hagen K; Hultcrantz R Gastroenterologiskt Centrum, Huddinge Sjukhus.

Lakartidningen (Sweden) Aug 5 1998, 95 (32-33) p3430-5

Genetic haemochromatosis (GH) is one of the most common hereditary diseases, with a prevalence of 1-5/1000 in the Western world. In 90 per cent of cases a mutation is found in an MHC-class-like gene designated HFE, involving a substitution at position 282 of the HFE protein and resulting in defective binding of beta(2)-microglobulin. Animals with beta(2)-microglobulin deficiency develop iron overload, indicating this protein to be involved in the regulation of iron metabolism. Hepatic iron overload results in increased production of oxygen free radicals and peroxidation of membrane lipids, thus causing damage to lysosomes, mitochondria and the endoplasmic reticulum. These cellular events may progress to cell death, fibrogenesis, and the development of liver cirrhosis which is associated with a 200-fold increase in risk of hepatocellular carcinoma. In addition to the risk of diabetes, arthralgia, cardiac arrhythmia, pituitary insufficiency and hypogonadism, iron excess is also associated with aggravation of the cytotoxic effects exerted on hepatocytes by other agents such as alcohol or hepatotropic viruses. The treatment of iron overload in GH consists of weekly venesection until the serum ferritin level is normalized, followed by maintenance therapy. Survival rates are normal if the disease is detected and treated before complications have developed. (45 Refs.)

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Haemochromatosis.

Worwood M Department of Haematology, University of Wales College of Medicine, Cardiff, UK.

Clin Lab Haematol (England) Apr 1998, 20 (2) p65-75

Genetic haemochromatosis (GH) is the most common, autosomal recessive disorder in Northern Europe. The studies which led to the identification of the HFE gene are described. In the UK over 90% of patients with GH are homozygous for the C282Y mutation of this gene. This mutation is confined to populations of European origin. The significance of another mutation, H63D, in causing iron overload is less certain. Preliminary studies on the localization of the protein and the effects of the mutations are described. Genetic testing and the measurement of iron status now provide the means to allow for widespread testing for the prevention of iron overload and its consequences. However, questions remain about the clinical penetrance of GH. (81 Refs.)

Hemochromatosis and iron needs.

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Nutr Rev (UNITED STATES) Feb 1998, 56 (2 Pt 2) ps30-7; discussion s54-75

Although iron is an essential dietary requirement, the amount absorbed by the body is well regulated and depends on body iron stores and on dietary iron availability. There is very little iron excreted under normal conditions. Iron deficiency is a worldwide problem but iron overload, as seen in the inherited disease, hemochromatosis, is a major cause of morbidity in some Caucasian populations. This is a problem particularly where there is an adequate dietary iron intake and especially in males. A mutation has recently been described in an MHC Class I-like gene (HFE) that encodes for a protein (HFE) of 343 amino acids. The molecule contains a signal sequence peptide-binding region, alpha, and alpha(2) domains, and an immunoglobulin-like alpha(3) domain, in addition to a transmembrane region and a small cytoplasmic tail. It is a candidate gene for hemochromatosis. Several possibilities as to the function of this gene and the corresponding protein have been suggested but none has yet been confirmed. The mutation has been detected by several different groups in 80%-100% of subjects with the disease. However, in one study, 18%-20% of patients with the mutation did not exhibit significant iron overload. The discovery of this gene has important implications for both clinical studies and the elucidation of the pathways of iron metabolism. (41 Refs.)

Long-term intraperitoneal deferoxamine for hemochromatosis

Swartz R.D.; Legault D.J. Michigan University Medical Center, 3914 TC-Box 0364, Ann Arbor, MI 48109-0364 USA

American Journal of Medicine (USA), 1996, 100/3 (308-312)

Intraperitoneal deferoxamine is a well-established treatment for aluminum accumulation syndrome in patients with end-stage renal disease receiving peritoneal dialysis, but the use of intraperitoneal deferoxamine has not been described outside of the setting of chronic renal failure. We present here a case of secondary hemochromatosis, complicated by cirrhosis and cardiomyopathy, in which a chronic peritoneal dialysis catheter was used both to treat ascites and to deliver parenteral deferoxamine for iron overload. Daily urinary iron excretion was similar to that achieved when using standard routes of deferoxamine administration. Over a 2-year period, reversal of both the biochemical indicators and the clinical manifestations of iron overload was accomplished.

Antioxidant activity of Vitamin-C in iron-overloaded human plasma

Berger T.M.; Polidori M.C.; Dabbagh A.; Evans P.J.; Halliwell B.; Morrow J.D.; Roberts II L.J.; Frei B. B. Frei, Whitaker Cardiovascular Inst., Boston University School of Medicine, 80 East Concord St., Boston, MA 02118 USA

Journal of Biological Chemistry (USA), 1997, 272/25 (15656-15660)

Vitamin-C (ascorbic acid, AA) can act as an antioxidant or a pro-oxidant in vitro, depending on the absence or the presence, respectively, of redox-active metal ions. Some adults with iron-overload and some premature infants have potentially redox-active, bleomycin-detectable iron (BDI) in their plasma. Thus, it has been hypothesized that the combination of AA and BDI causes oxidative damage in vivo. We found that plasma of preterm infants contains high levels of AA and F2-isoprostanes, stable lipid peroxidation end products. However, F2-isoprostane levels were not different between those infants with BDI (138 plus or minus 51 pg/ml, n = 19) and those without (126 plus or minus 41 pg/ml, n = 10), and the same was true for protein carbonyls, a marker of protein oxidation (0.77 plus or minus 0.31 and 0.68 plus or minus 0.13 nmol/mg protein, respectively). Incubation of BDI-containing plasma from preterm infants did not result in detectable lipid hydroperoxide formation (less than or equal to 10 nM cholesteryl ester hydroperoxides) as long as AA concentrations remained high. Furthermore, when excess iron was added to adult plasma, BDI became detectable, and endogenous AA was rapidly oxidized. Despite this apparent interaction between excess iron and endogenous AA, there was no detectable lipid peroxidation as long as AA was present at >10% of its initial concentration. Finally, when iron was added to plasma devoid of AA, lipid hydroperoxides were formed immediately, whereas endogenous and exogenous AA delayed the onset of iron-induced lipid peroxidation in a dose-dependent manner. These findings demonstrate that in iron-overloaded plasma, AA acts as an antioxidant toward lipids. Furthermore, our data do not support the hypothesis that the combination of high plasma concentrations of AA and BDI, or BDI alone, causes oxidative damage to lipids and proteins in vivo.

Antioxidant status and lipid peroxidation in hereditary haemochromatosis.

Young IS; Trouton TG; Torney JJ; McMaster D; Callender ME; Trimble ER Department of Clinical Biochemistry, Queen's University of Belfast, UK.

Free Radic Biol Med (United States) Mar 1994, 16 (3) p393-7

Hereditary haemochromatosis is characterised by iron overload that may lead to tissue damage. Free iron is a potent promoter of hydroxyl radical formation that can cause increased lipid peroxidation and depletion of chain-breaking antioxidants. We have therefore assessed lipid peroxidation and antioxidant status in 15 subjects with hereditary haemochromatosis and age/sex matched controls. Subjects with haemochromatosis had increased serum iron (24.8 (19.1-30.5) vs. 17.8 (16.1-19.5) $\mu\text{mol/l}$, $p = 0.021$) and % saturation (51.8 (42.0-61.6) vs. 38.1 (32.8-44.0), $p = 0.025$). Thiobarbituric acid reactive substances (TBARS), a marker of lipid peroxidation, were increased in haemochromatosis (0.59 (0.48-0.70) vs. 0.46 (0.21-0.71) $\mu\text{mol/l}$, $p = 0.045$), and there were decreased levels of the chain-breaking antioxidants alpha-tocopherol (5.91 (5.17-6.60) vs. 7.24 (6.49-7.80) $\mu\text{mol/mmol}$ cholesterol, $p = 0.001$), ascorbate (51.3 (33.7-69.0) vs. 89.1 (65.3-112.9), $p = 0.013$), and retinol (1.78 (1.46-2.10) vs. 2.46 (2.22-2.70) $\mu\text{mol/l}$, $p = 0.001$). Patients with hereditary haemochromatosis have reduced levels of antioxidant vitamins, and nutritional antioxidant supplementation may represent a novel approach to preventing tissue damage. However, the use of Vitamin-C may be deleterious in this setting as ascorbate can have prooxidant effects in the presence of iron overload.

Iron storage, lipid peroxidation and glutathione turnover in chronic anti-HCV positive hepatitis.

Farinati F, Cardin R, De Maria N, Della Libera G, Marafin C, Lecis E, Burra P, Floreani A, Cecchetto A, Naccarato R Cattedra Malattie Apparato Digerente, Universita di Padova, Italy.

J Hepatol 1995 Apr;22(4):449-56

BACKGROUND/AIMS: Little is known about the pathogenesis of liver damage related to hepatitis C virus. The presence of steatosis or increased ferritin levels, and preliminary data on the relevance of iron as a prognostic factor prompted us to ascertain whether hepatitis C virus-related liver damage might be mediated by iron accumulation.

METHODS: We evaluated the degree of hepatic inflammation and steatosis, serum ferritin, transferrin saturation and iron levels, tissue iron concentrations and iron index, liver glutathione and malondialdehyde in 33 males and 20 females with chronic hepatitis C virus- or hepatitis B virus-related hepatitis (42 + 11). We also considered six patients with both alcohol abuse and hepatitis C virus, four males with chronic alcoholic liver disease and four males with genetic hemochromatosis, giving a total of 67. All diagnoses were histologically confirmed. Patients with cirrhosis were excluded.

RESULTS: Our data show that: 1. Steatosis is more frequent in hepatitis C virus and hepatitis C virus+alcohol abuse patients; 2. In males, serum ferritin and tissue iron are significantly higher in hepatitis C virus- than in hepatitis B virus-positive patients ($p < 0.01$ and 0.05); transferrin saturation is higher ($p < 0.05$) in hepatitis C virus-positive than in hepatitis B virus-positive patients only when males and females are considered together; 3. Serum ferritin and transferrin saturation only correlate with liver iron ($r = 0.833$ and $r = 0.695$, respectively, $p = 0.00001$); tissue iron is significantly higher in hepatitis C virus- than in hepatitis B virus-positive patients ($p < 0.05$); 4. In patients with chronic hepatitis, serum ferritin is a better marker of liver iron storage than transferrin saturation, both in males and in females; 5. Hepatitis C virus-positive patients have higher malondialdehyde levels and activation of turnover of glutathione, probably in response to free-radical-mediated liver damage. Females have lower liver iron levels but similar trends.















CONCLUSIONS: These findings suggest that hepatitis C virus-related liver damage is characterized by increased iron storage (possibly induced by the virus) which elicits a free-radical-mediated peroxidation, with consequent steatosis and activation of glutathione turnover.

HEMOCHROMATOSIS

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Berger T.M.; Polidori M.C.; Dabbagh A.; Evans P.J.; Halliwell B.; Morrow J.D.; Roberts II L.J.; Frei B.
B. Frei, Whitaker Cardiovascular Inst., Boston University School of Medicine, 80 East Concord St., Boston, MA 02118 USA
Journal of Biological Chemistry (USA), 1997, 272/25 (15656-15660)

Vitamin-C (ascorbic acid, AA) can act as an antioxidant or a pro-oxidant in vitro, depending on the absence or the presence, respectively, of redox-active metal ions. Some adults with iron-overload and some premature infants have potentially redox-active, bleomycin-detectable iron (BDI) in their plasma. Thus, it has been hypothesized that the combination of AA and BDI causes oxidative damage in vivo. We found that plasma of preterm infants contains high levels of AA and F2-isoprostanes, stable lipid peroxidation end products. However, F2-isoprostane levels were not different between those infants with BDI (138 plus or minus 51 pg/ml, n = 19) and those without (126 plus or minus 41 pg/ml, n = 10), and the same was true for protein carbonyls, a marker of protein oxidation (0.77 plus or minus 0.31 and 0.68 plus or minus 0.13 nmol/mg protein, respectively). Incubation of BDI-containing plasma from preterm infants did not result in detectable lipid hydroperoxide formation (less than or equal to 10 nM cholesteryl ester hydroperoxides) as long as AA concentrations remained high. Furthermore, when excess iron was added to adult plasma, BDI became detectable, and endogenous AA was rapidly oxidized. Despite this apparent interaction between excess iron and endogenous AA, there was no detectable lipid peroxidation as long as AA was present at >10% of its initial concentration. Finally, when iron was added to plasma devoid of AA, lipid hydroperoxides were formed immediately, whereas endogenous and exogenous AA delayed the onset of iron-induced lipid peroxidation in a dose-dependent manner. These findings demonstrate that in iron-overloaded plasma, AA acts as an antioxidant toward lipids. Furthermore, our data do not support the hypothesis that the combination of high plasma concentrations of AA and BDI, or BDI alone, causes oxidative damage to lipids and proteins in vivo.

Effect of vitamin E supplementation on hepatic fibrogenesis in chronic dietary iron overload

Brown K.E.; Poulos J.E.; Li L.; Soweid A.M.; Ramm G.A.; O'Neill R.; Britton R.S.; Bacon B.R.
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American Journal of Physiology - Gastrointestinal and Liver Physiology (USA), 1997, 272/1 35-1 (G116-G123)

It has been suggested that lipid peroxidation plays an important role in hepatic fibrogenesis resulting from chronic iron overload. Vitamin E is an important lipid-soluble antioxidant that has been shown to be decreased in patients with hereditary hemochromatosis and in experimental iron overload. The aim of this study was to determine the effects of vitamin E supplementation on hepatic lipid peroxidation and fibrogenesis in an animal model of chronic iron overload. Rats were fed the following diets for 4, 8, or 14 mo: standard laboratory diet (control), diet with supplemental vitamin E (200 IU/kg, control + E), diet with carbonyl iron (Fe), and diet with carbonyl iron supplemented with vitamin E (200 IU/kg, Fe + E). Iron loading resulted in significant decreases in hepatic and plasma vitamin E levels at all time points, which were overcome by vitamin E supplementation. Thiobarbituric acid-reactive substances (an index of lipid peroxidation) were increased three- to fivefold in the iron-loaded livers; supplementation with vitamin E reduced these levels by at least 50% at all time points. Hepatic hydroxyproline levels were increased twofold by iron loading. Vitamin E did not affect hydroxyproline content at 4 or 8 mo but caused an 18% reduction at 14 mo in iron-loaded livers. At 8 and 14 mo, vitamin E decreased the number of alpha-smooth muscle actin-positive stellate cells in iron-loaded livers. These results demonstrate a dissociation between lipid peroxidation and collagen production and suggest that the profibrogenic action of iron in this model is mediated through effects which cannot be completely suppressed by vitamin E.

Iron in liver diseases other than hemochromatosis

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Seminars in Liver Disease (USA), 1996, 16/1 (65-82)

There is growing evidence that normal or only mildly increased amounts of iron in the liver can be damaging, particularly when they are combined with other hepatotoxic factors such as alcohol, porphyrogenic drugs, or chronic viral hepatitis. Iron enhances the pathogenicity of microorganisms, adversely affects the function of macrophages and lymphocytes, and enhances fibrogenic pathways, all of which may increase hepatic injury due to iron itself or to iron and other factors. Iron may also be a co-carcinogen or promoter of hepatocellular carcinoma, even in patients without HC or cirrhosis. Based on this and other evidence, we hope that the era of indiscriminate iron supplementation will come to an end. Bloodletting, a therapy much in vogue 2 centuries ago, is deservedly enjoying a renaissance, based on our current understanding of the toxic effects of iron and the benefits of its depletion.

Metal-induced hepatotoxicity

Britton R.S.

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Seminars in Liver Disease (USA), 1996, 16/1 (3-12)

Figure 3 summarizes several proposed mechanisms of iron- or copper- induced hepatotoxicity. It has long been suspected that free radicals may play a role in iron- and copper-induced cell toxicity because of the powerful prooxidant action of iron and copper salts in vitro. In the presence of available cellular reductants, iron or copper in low molecular weight forms may play a catalytic role in the initiation of free radical reactions. The resulting oxyradicals have the potential to damage cellular lipids, nucleic acids, proteins, and carbohydrates, resulting in wide-ranging impairment in cellular function and integrity. However, cells are endowed with cytoprotective mechanisms (antioxidants, scavenging enzymes, repair processes) that act to counteract the effects of free radical production. Thus, the net effect of metal-induced free radicals on cellular function will depend on the balance between radical production and the cytoprotective systems. As a result, there may be a rate of free radical production that must be exceeded before cellular injury occurs. Evidence has now accumulated that iron or copper overload in experimental animals can result in oxidative damage to lipids in vivo, once the concentration of the metal exceeds a threshold level. In the liver, this lipid peroxidation is associated with impairment of membrane dependent functions of mitochondria (oxidative metabolism) and lysosomes (membrane integrity, fluidity, pH). Although these findings do not prove causality, it seems likely that lipid peroxidation is involved, since similar functional defects are produced by metal-induced lipid peroxidation in these organelles in vitro. Both iron and copper overload impair hepatic mitochondrial respiration, primarily through a decrease in cytochrome c oxidase activity. In iron overload, hepatocellular calcium homeostasis may be impaired through damage to mitochondrial and microsomal calcium sequestration. DNA has also been reported to be a target of metal-induced damage in the liver; this may have consequences as regards malignant transformation. The levels of some antioxidants in the liver are decreased in rats with iron or copper overload, which is also suggestive of ongoing oxidative stress. Reduced cellular ATP levels, lysosomal fragility, impaired cellular calcium homeostasis, and damage to DNA may all contribute to hepatocellular injury in iron and copper overload. There are few data addressing the key issue of whether free radical production is increased in patients with iron or copper overload. Patients with hereditary hemochromatosis have elevated plasma levels of TBA-reactants and increased hepatic levels of MDA-protein and HNE-protein adducts, indicative of lipid peroxidation. Mitochondria isolated from the livers of Wilson disease patients have evidence of lipid peroxidation, and some patients with Wilson disease have decreased hepatic and plasma levels of vitamin E. Additional investigation will be required to fully assess oxidant stress and its potential pathophysiologic role in patients with iron or copper overload.

Hepatocyte proliferative activity in chronic liver damage as assessed by the monoclonal antibody MIB1 Ki67 in archival material: The role of etiology, disease activity, iron, and lipid peroxidation

Farinati F.; Cardin R.; D'Errico A.; De Maria N.; Naccarato R.; Cecchetto A.; Grigioni W.

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Hepatology (USA), 1996, 23/6 (1468-1475)

Hepatitis B virus (HBV)- and hepatitis C virus (HCV)-related liver damage is linked to an increased risk of hepatocellular carcinoma, but the mechanisms underlying hepatitis C viral activity are not known. We therefore compared hepatocellular proliferative activity in chronic C virus-related hepatitis and in liver damage of other etiology. Hepatocyte proliferation rate was investigated in 56 patients with chronic hepatitis using the Ki67 MIB1 monoclonal antibody in archival material. According to etiology, the patients were subgrouped as follows: HCV (34), HBV (11), Alcohol (4), HCV + Alcohol (4), and Hemochromatosis (3). Proliferation rate was correlated with age, sex, etiology, disease activity, liver iron storage, free-radical production, and glutathione levels by regression and discriminant analysis. HCV-positive patients had significantly more MIB1-positive hepatocytes in the periportal area ($P < .011$) and in the low-proliferating perivenular area (zones 2 and 3) ($P < .05$). The number of MIB1-positive cells correlated directly with alanine transaminase (ALT) levels, Knodell index (KI), and, inversely, with iron saturation. By stepwise discriminant analysis, ALT levels and etiology were identified as single independent variables. These data suggest that HCV infection induces increased and abnormal hepatocyte proliferation, which might be related to the increased risk of hepatocellular carcinoma in patients with HCV-related liver damage.

Hepatic iron deposition in human disease and animal models

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BioMetals (United Kingdom), 1996, 9/2 (205-209)

Iron deposition occurs in parenchymal cells of the liver in two major defects in human subjects (i) in primary iron overload (genetic haemochromatosis) and (ii) secondary to anaemias in which erythropoiesis is increased (thalassaemia). Transfusional iron overload results in excessive storage primarily in cells of the reticulo endothelial system. The storage patterns in these situations are quite characteristic. Excessive iron storage, particularly in parenchymal cells eventually results in fibrosis and cirrhosis. There is no animal model of iron overload which completely mimics genetic haemochromatosis but dietary iron loading with carbonyl iron or ferrocene does produce excessive parenchymal iron stores in the rat. Such models have been used to study iron toxicity and the action of iron chelators in the effective removal of excessive iron stores.

Long-term intraperitoneal deferoxamine for hemochromatosis

Swartz R.D.; Legault D.J.

Michigan University Medical Center, 3914 TC-Box 0364, Ann Arbor, MI 48109-0364 USA

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Biological markers of oxidative stress induced by ethanol and iron overload in rat.

Wisniewska-Knypl JM; Wronska-Nofer T

Department of Toxicological Biochemistry, Nofer Institute of Occupational Medicine, Lodz, Poland.

Int J Occup Med Environ Health (Poland) 1994, 7 (4) p355-63

Studies on rats treated for 15 months with ethanol (10%, w/v, solution in drinking water) revealed that the stimulation of hepatic cytochrome P-450 monooxygenases activity was accompanied by enhanced microsomal malondialdehyde formation, a lipid peroxidation index and a decreased level of the antioxidant, alpha-tocopherol. The other components of the prooxidant/antioxidant system, diene conjugates and catalase, glutathione peroxidase and superoxide dismutase activities were unaffected. Oxidative stress in blood was shown by a significant decrease in the alpha-tocopherol level whereas lipid peroxidation and antioxidant enzyme activity remained unchanged. The prooxidative effect of ethanol was catalytically promoted by an iron overload (Fe-saccharate, 100 mg Fe³⁺/kg body wt. intraperitoneally, 2, 5 and 7 day before test) to simulate the effect of alcoholic hemochromatosis. Thus, the level of malondialdehyde and alpha-tocopherol in the serum may be recommended as biological markers of ethanol-provoked oxidative stress, which is especially useful in the evaluation of the combined effect of ethanol and other chemicals that affect the redistribution of active iron complexes.

Antioxidant status and lipid peroxidation in hereditary haemochromatosis.

Young IS; Trouton TG; Torney JJ; McMaster D; Callender ME; Trimble ER

Department of Clinical Biochemistry, Queen's University of Belfast, UK.

Free Radic Biol Med (United States) Mar 1994, 16 (3) p393-7

Hereditary haemochromatosis is characterised by iron overload that may lead to tissue damage. Free iron is a potent promoter of hydroxyl radical formation that can cause increased lipid peroxidation and depletion of chain-breaking antioxidants. We have therefore assessed lipid peroxidation and antioxidant status in 15 subjects with hereditary haemochromatosis and age/sex matched controls. Subjects with haemochromatosis had increased serum iron (24.8 (19.1-30.5) vs. 17.8 (16.1-19.5) $\mu\text{mol/l}$, $p = 0.021$) and % saturation (51.8 (42.0-61.6) vs. 38.1 (32.8-44.0), $p = 0.025$). Thiobarbituric acid reactive substances (TBARS), a marker of lipid peroxidation, were increased in haemochromatosis (0.59 (0.48-0.70) vs. 0.46 (0.21-0.71) $\mu\text{mol/l}$, $p = 0.045$), and there were decreased levels of the chain-breaking antioxidants alpha-tocopherol (5.91 (5.17-6.60) vs. 7.24 (6.49-7.80) $\mu\text{mol/mmol}$ cholesterol, $p = 0.001$), ascorbate (51.3 (33.7-69.0) vs. 89.1 (65.3-112.9), $p = 0.013$), and retinol (1.78 (1.46-2.10) vs. 2.46 (2.22-2.70) $\mu\text{mol/l}$, $p = 0.001$). Patients with hereditary haemochromatosis have reduced levels of antioxidant vitamins, and nutritional antioxidant supplementation may represent a novel approach to preventing tissue damage. However, the use of Vitamin-C may be deleterious in this setting as ascorbate can have prooxidant effects in the presence of iron overload.

Iron storage, lipid peroxidation and glutathione turnover in chronic anti-HCV positive hepatitis.

Farinati F, Cardin R, De Maria N, Della Libera G, Marafin C, Lecis E, Burra P, Floreani A, Cecchetto A, Naccarato R
Cattedra Malattie Apparato Digerente, Universita di Padova, Italy.
J Hepatol 1995 Apr;22(4):449-56

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METHODS: We evaluated the degree of hepatic inflammation and steatosis, serum ferritin, transferrin saturation and iron levels, tissue iron concentrations and iron index, liver glutathione and malondialdehyde in 33 males and 20 females with chronic hepatitis C virus- or hepatitis B virus-related hepatitis (42 + 11). We also considered six patients with both alcohol abuse and hepatitis C virus, four males with chronic alcoholic liver disease and four males with genetic hemochromatosis, giving a total of 67. All diagnoses were histologically confirmed. Patients with cirrhosis were excluded.

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Induction of oxidative single- and double-strand breaks in DNA by ferric citrate.

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Molecular Biology Branch, Center for Devices and Radiological Health, Food and Drug Administration, Rockville, MD 20857.
Free Radic Biol Med (United States) Aug 1993, 15 (2) p117-23

The relative risk of primary hepatocellular carcinoma in genetic hemochromatosis (GH) is estimated at over 200 times as that of control populations. Recently, ferric ion chelated to citrate (Fe-citrate) was identified as the major non-transferrin-bound iron in the serum of GH patients. We investigated whether low concentration of Fe-citrate plus reductant could damage supercoiled plasmid DNA under physiological pH and ionic strength. Incubation of Fe-citrate with either H₂O₂, L-ascorbate, or L-cysteine induced single- and double-strand breaks in supercoiled plasmid pZ189 in a concentration- and time-dependent fashion. DNA strand breaks produced by Fe-citrate plus H₂O₂ increased at reduced pH ($< \text{or} = 6.9$). Catalase and free radical scavengers inhibited the DNA breakage produced by Fe-citrate in combination with each reductant, suggesting that H₂O₂ and finally .OH are responsible DNA damaging species. The catalytic ability of Fe-citrate to induce DNA strand breaks, particularly double-strand breaks (DSBs), may contribute to the carcinogenic processes observed in GH.

A unique rodent model for both the cardiotoxic and hepatotoxic effects of prolonged iron overload.

Carthew P, Dorman BM, Edwards RE, Francis JE, Smith AG
MRC Toxicology Unit, University of Leicester, United Kingdom.
Lab Invest 1993 Aug;69(2):217-22

BACKGROUND: Hemochromatosis is a disease of excessive iron storage leading to tissue damage and fibrosis. Both genetic hemochromatosis, which can affect 1 in 500 of some populations, and the form of this disease which occurs as a secondary consequence of the hemoglobinopathy, homozygous beta-thalassemia, with 40 million carriers worldwide, have a common pathology. The cardiotoxicity and hepatotoxicity, which occurs with this disease, have never been produced experimentally in other species.

EXPERIMENTAL DESIGN: Using a regimen of iron dextran administered subcutaneously to gerbils on a weekly basis for 7 weeks, we have produced severe hemosiderosis, especially of the liver and heart. By examining gerbils at 1, 2 and 3 months after the final iron injections we followed the subsequent development of hemochromatosis in the hearts and livers of iron overloaded animals.

RESULTS: Hemochromatosis of the liver was evident as a scarring fibrosis in all cases between 1 and 3 months after iron dextran administration to gerbils. The iron burden in the cardiac myocytes of gerbils gradually increased between 1 and 3 months, resulting in hemochromatosis of the heart 2 and 3 months after the final iron dextran injections.

CONCLUSIONS: Repeated parenteral injections of iron dextran to gerbils resulted in hemochromatosis affecting the liver and heart with a pathology which is the same as occurs in the end-stage disease in man. This model will allow the detailed study of the mechanism of iron induced, free radical tissue damage, which is thought to be the cause of these lesions and will also be useful in the evaluation of iron chelating therapies to determine whether the hepatic and cardiac pathology of iron overload can be modulated over a long period.

Biochemical and biophysical investigations of the ferrocene-iron-loaded rat. An animal model of primary haemochromatosis.

Ward RJ; Florence AL; Baldwin D; Abiaka C; Roland F; Ramsey MH; Dickson DP; Peters TJ; Crichton RR
Department of Clinical Biochemistry, King's College School of Medicine and Dentistry, London, England.
Eur J Biochem (Germany) Dec 5 1991, 202 (2) p405-10

Male Wistar rats fed with ferrocene had high hepatic iron loading (7.24 +/- 1.97 mg Fe/g tissue) after 6 weeks, principally located in lysosomes, which was comparable to the levels and distribution determined in human haemochromatosis. The two iron-storage proteins, ferritin and haemosiderin were isolated from the livers of the ferrocene-loaded rats and their iron cores were investigated by Mossbauer spectroscopy and inductively coupled plasma-emission spectrometry. Ferrihydrite was the predominant form of iron present in both ferritin and haemosiderin, while haemosiderin contained higher amounts of phosphorus, magnesium, calcium and barium, than either normal or ferrocene-loaded ferritin. Free-radical-mediated damage in the iron-loaded livers was inferred by the significant depletion of alpha-tocopherol in both the livers and subcellular hepatic lysosomal fraction, which inversely correlated with the increasing iron content ($r = -0.61$; P less than 0.05) and was associated with increased fragility of the lysosomal membranes.

Antioxidant and iron-chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures

Morel I, Lescoat G, Cogrel P, Sergent O, Padeloup N, Brissot P, Cillard P, Cillard J
Laboratoire de Biologie Cellulaire et Vegetale, UFR des Sciences Pharmaceutiques, Rennes, France.
Biochem Pharmacol 1993 Jan 7;45(1):13-9

The cytoprotective effect of three flavonoids, catechin, quercetin and diosmetin, was investigated on iron-loaded hepatocyte cultures, considering two parameters: the prevention of iron-increased lipid peroxidation and the inhibition of intracellular enzyme release. These two criteria of cytoprotection allowed the calculation of mean inhibitory concentrations (IC50) which revealed that the effectiveness of these flavonoids could be classified as follows: catechin>quercetin>diosmetin. These IC50 values have been related to structural characteristics of the flavonoids tested. Moreover, the investigation of the capacity of these flavonoids to remove iron from iron-loaded hepatocytes revealed a good relationship between this iron-chelating ability and the cytoprotective effect. The cytoprotective activity of catechin, quercetin and diosmetin could thus be ascribed to their widely known antiradical property but also to their iron-chelating effectiveness. These findings increase further the prospects for the development and clinical application of these potent antioxidants.

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The effects of caffeic acid and its related catechols on hydroxyl radical formation by 3-hydroxyanthranilic acid, ferric chloride, and hydrogen peroxide

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Department of Chemistry, Wakayama Medical College, Japan.
Arch Biochem Biophys 1990 Jan;276(1):242-7

The effect of caffeic acid on hydroxyl radical formation through a reaction, which contained 0.22 M carbonate buffer (pH 7.4), 0.22 mM 3-hydroxyanthranilic acid, 87 mM 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), 2.9 mM hydrogen peroxide, and 14 microM FeCl₃, was investigated. The addition of 30 microM caffeic acid resulted in the decrease of hydroxyl radical formation in the reaction mixture. Chlorogenic acid, 3,4-dihydroxy-phenylalanine noradrenaline, gallic acid, dopamine, epicatechin, and D-(+)-catechin also suppressed the hydroxyl radical formation. In regard to the positional isomers of benzenediol, o-benzenediol inhibited the hydroxyl radical formation, but m- and p-benzenediol did not. The inhibitory effect of the hydroxyl radical formation seems to be due to the chelation of iron ions by the catechols. Supporting evidence includes the diminished effect of catechols in the presence of EDTA (a potent iron ion chelator) and the observation of a visible band at 450 nm caused by the interaction between caffeic acid and iron ions. Additionally, the visible band (506 nm) was observed in the solution of o-benzenediol and ferric chloride but not in the solution of m- or p-benzenediol and ferric chloride. Thus compounds with adjacent hydroxyl groups on aromatic rings might inhibit hydroxyl radical formation.

A novel antioxidant flavonoid (IdB 1031) affecting molecular mechanisms of cellular activation

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Free Radic. Biol. Med. (USA), 1994, 16/5 (547-553)

In searching for new drug candidates which could help bridge the gaps between free radical oxidations, pathophysiological responses, and pharmacological treatment, a series of flavonoids was screened. The most interesting compound emerging from this screening, the flavone 3'-hydroxyfarrerol (IdB 1031), is presented in this article. This compound is a good inhibitor of microsomal lipid peroxidation induced by either iron-adenosine 5'-diphosphate (ADP) or carbon tetrachloride. The elevated rate constant for the interaction with peroxy radicals, analysed by the kinetics of inhibition of crocin bleaching in the presence of a diazo initiator, gives an account for the observed antioxidant capacity. When tested on human neutrophils activated by fMLP, IdB 1031 inhibits (ID₅₀:20 microM) respiratory burst. This effect, which is possibly linked to the observed inhibition of protein-kinase C (ID₅₀:50 microM), seems rather specific since IdB 1031 does not inhibit tyr-kinases and casein-kinase-2, while Quercetin and other flavonoids inhibit unspecifically all these enzymes. These effects, as a whole, depict this compound as a drug candidate for diseases in which peroxidative damage is associated with the induction of inflammatory responses and specifically with activation of a respiratory burst of leucocytes.

Prevention of postischemic cardiac injury by the orally active iron chelator 1,2-dimethyl-3-hydroxy-4-pyridone (L1) and the antioxidant (+)-cyanidanol-3

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In this study, we investigated the role of oxygen-derived free radicals and iron in mediating myocardial injury during ischemia and reperfusion. Iron is of special interest because it may enhance tissue injury during ischemia and reperfusion by catalyzing the formation of highly reactive hydroxyl radicals (by modified Haber-Weiss or Fenton reactions). Rat hearts, perfused by the Langendorff method, were subjected to global ischemia (15 minutes at 37°C) and reperfusion. The effects of two iron chelators, 1,2-dimethyl-3-hydroxy-4-pyridone (L1) and 5-hydroxy-2-hydroxymethyl-4-pyrone (kojic acid), and one antioxidant, (+)-cyanidanol-3, on contractile function, coronary flow, lactate dehydrogenase release, and lactate production were studied. The combination of these iron chelators is of special importance because L1 is known to prevent lipid peroxidation, induced by ADP/Fe³⁺ and NADPH in microsomes, in contrast to kojic acid. We found significant protection of contractile function (apex displacement) during reperfusion with 50 µM L1 and 20 µM (+)-cyanidanol-3 ($p < 0.01$, $n = 6$), whereas no protection was found with 50 µM kojic acid ($n = 6$). Measurements of lactate dehydrogenase release during reperfusion showed a protective pattern similar to that found for heart contractile function, although 50 µM kojic acid also showed a significantly lower lactate dehydrogenase release during the first 10 minutes of reperfusion. No differences in coronary resistance or lactate release were found between the various groups. Our findings indicate that iron and oxygen-derived free radicals are important in the pathogenesis of postischemic reperfusion injury probably because of the formation of hydroxyl radicals. During heart ischemia, administration of the orally active iron chelator L1 or the antioxidant (+)-cyanidanol-3 may be a promising approach in establishing postischemic cardiac protection.

Hepatotoxicity of menadione predominates in oxygen-rich zones of the liver lobule

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J. Pharmacol. Exp. Ther. (USA), 1989, 248/3 (1317-1322)

This study was designed to investigate the mechanism of zone-specific hepatotoxicity due to menadione. Infusion of menadione (64-1000 µM) into perfused livers from fasted rats caused a concentration-dependent increase in O₂ uptake. During perfusion in the anterograde direction, menadione (1 mM) increased O₂ uptake from 115 plus or minus 11 to 142 plus or minus 10 micromol/g/hr within 30 min, followed by a decrease to 92 plus or minus 11 micromol/g/hr over the next 30 min. Trypan blue was taken up by 90% of cells in periportal regions reflecting irreversible cell death, whereas cells in pericentral areas were not damaged. When the hepatic O₂ gradient was reversed by perfusing in the retrograde direction, menadione increased O₂ uptake initially from 114 plus or minus 11 to 132 plus or minus 14 micromol/g/hr, followed by a decline to 51 plus or minus 12 micromol/g/hr, qualitatively similar to data obtained from perfusions in the natural, anterograde direction. During perfusions in the retrograde direction, however, 95% of cells in pericentral regions were stained with trypan blue whereas those in periportal areas were spared. O₂ uptake in specific zones of the liver lobule was then measured with miniature O₂ electrodes. When menadione was infused during anterograde perfusions, O₂ uptake increased in O₂-rich periportal areas from 128 plus or minus 6 to 156 plus or minus 12 micromol/g/hr, but was not altered in pericentral regions. Conversely, during perfusions in the retrograde direction, menadione did not affect O₂ uptake in periportal areas, but stimulated uptake in O₂-rich pericentral regions from 120 plus or minus 4 to 150 plus or minus 14 micromol/g/hr. Lowering the O₂ tension across the lobule by perfusing with buffer saturated with 21% O₂ prevented menadione-induced lactate dehydrogenase release and uptake of trypan blue. Thus, menadione increases O₂ uptake and damages cells nearly exclusively in O₂-rich regions of the liver lobule. Lactate dehydrogenase release and trypan blue uptake due to menadione were prevented by cyanidanol (400 µM), a radical scavenger, and allopurinol (1 mM), an inhibitor of xanthine oxidase. Desferrioxamine (100 µM), an iron chelator, prevented trypan blue uptake due to menadione and reduced enzyme release by 38%. Taken together, these results indicate that menadione is an O₂-dependent hepatotoxin which acts via the production of radical species.

Iron-load increases the susceptibility of rat hearts to oxygen reperfusion damage. Protection by the antioxidant (+)-cyanidanol-3 and deferoxamine

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Circulation (USA), 1988, 78/2 (442-449)

To investigate whether iron is involved in the reperfusion syndrome by aggravating free radical injury, the hearts from iron-loaded and control rats were perfused under normoxic, anoxic, and reperfusion conditions. Normoxic perfusion revealed no change in coronary flow, contractility, or lactate dehydrogenase (LDH) release between these two groups. Under anoxic and reperfusion conditions, however, we found a significant increase of ventricle fibrillation (56% vs. 0%, $p < 0.01$, $n = 9$), a significantly lower recovery of contractility (21 plus or minus 7.4% vs 81 plus or minus 6.6%, mean plus or minus SEM; $p < 0.001$), and a significant increase of LDH release (667 plus or minus 142 vs. 268 plus or minus 37 mU LDH/min/g wet wt, mean plus or minus SEM; $p < 0.05$).

Administration of either 20 microM of the antioxidant (+)-cyanidanol-3 or 50 microM of the iron-chelator deferoxamine totally prevented the generation of ventricle fibrillation and normalized contractility to control levels in the iron-loaded group. Moreover, 20 microM (+)-cyanidanol-3 significantly lowered LDH release in this period (312plus or minus67 mU), whereas deferoxamine had no protective effect on this LDH release (1,494plus or minus288 mU). Normal hearts appeared to be protected by 20 microM (+)-cyanidanol-3 as well. In this group (n=6), a significantly higher recovery of contractility (97.1plus or minus3.2% vs 81plus or minus6.6%, p<0.05) and a significantly lower release of LDH (110plus or minus27 vs. 268plus or minus37 mU, p<0.05) was found compared with the control group (n=9). No difference in superoxide dismutase or glutathione peroxidase activity was found between the groups. It is concluded that

1) iron-loaded rat hearts are more susceptible to anoxia and oxygen reperfusion damage;

2) iron load itself, under normoxic conditions, does not seem to be harmful; and

3) the antioxidant (+)-cyanidanol-3 is able to protect normal as well as iron-loaded hearts against anoxic and reperfusion damage. We suggest that iron plays an important role in the occurrence of tissue damage and ventricle fibrillation during anoxia and reperfusion, probably through the formation of hydroxyl radicals and/or perferryl oxide.

Hepatocyte injury resulting from the inhibition of mitochondrial respiration at low oxygen concentrations involves reductive stress and oxygen activation

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Chem Biol Interact 1995 Oct 20;98(1):27-44

By correlating lactate/pyruvate ratios and ATP levels, cytotoxicity induced by the mitochondrial respiratory inhibitors or hypoxia:reoxygenation injury can be attributed not only to ATP depletion but also to reductive stress and oxygen activation. Thus hypoxia, cyanide or antimycin markedly increases reductive stress, non-heme Fe release and H₂O₂ formation in hepatocytes. Cytotoxicity was partly prevented with the ferric chelator desferoxamine, the xanthine oxidase inhibitor oxypurinol and the hydrogen peroxide scavenger glutathione. No lipid peroxidation could be detected and phenolic antioxidants had little effect. However, polyphenolic antioxidants or the superoxide dismutase mimics TEMPO or TEMPOL partly prevented cytotoxicity. Furthermore, increasing the hepatocyte NADH/NAD⁺ ratio with NADH generating compounds such as ethanol, glycerol, or beta-hydroxybutyrate markedly increased cytotoxicity (prevented by desferoxamine) and further increased the intracellular release of non-heme iron. Cytotoxicity could be prevented by glycolytic substrates (eg. fructose, dihydroxyacetone, glyceraldehyde) or the NADH utilising substrates acetoacetate or acetaldehyde which decreased the reductive stress and prevented intracellular iron release. These results suggest that liver injury resulting from insufficient respiration involves reductive stress which releases intracellular Fe, converts xanthine dehydrogenase to xanthine oxidase and causes mitochondrial oxygen activation. The cell's antioxidant defences are compromised and ATP catabolism contributes to oxygen activation.

Modulating hypoxia-induced hepatocyte injury by affecting intracellular redox state

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Biochim Biophys Acta 1995 Nov 9;1269(2):153-61

Hypoxia-induced hepatocyte injury results not only from ATP depletion but also from reductive stress and oxygen activation. Thus the NADH/NAD⁺ ratio was markedly increased in isolated hepatocytes maintained under 95% N₂/5% CO₂ in Krebs-Henseleit buffer well before plasma membrane disruption occurred. Glycolytic nutrients fructose, dihydroxyacetone or glyceraldehyde prevented cytotoxicity, restored the NADH/NAD⁺ ratio, and prevented complete ATP depletion. However, the NADH generating nutrients sorbitol, xylitol, glycerol and beta-hydroxybutyrate enhanced hypoxic cytotoxicity even though ATP depletion was not affected. On the other hand, NADH oxidising metabolic intermediates oxaloacetate or acetoacetate prevented hypoxic cytotoxicity but did not affect ATP depletion. Restoring the cellular NADH/NAD⁺ ratio increased the intracellular release of iron. Hypoxia-induced hepatocyte injury was also prevented by oxypurinol, a xanthine oxidase inhibitor. Polyphenolic antioxidants (green tea extracts) or the superoxide dismutase mimic, TEMPO partly prevented cytotoxicity suggesting that reactive oxygen species contributed to the cytotoxicity. The above results suggests that hypoxia induced hepatocyte injury results from sustained reductive stress and oxygen activation.

Protection of rat myocardial phospholipid against peroxidative injury through superoxide-(xanthine oxidase)-dependent, iron-promoted fenton chemistry by the male contraceptive gossypol

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Biochem. Pharmacol. (United Kingdom), 1988, 37/17 (3335-3342)

Metal-promoted oxygen free-radical chemistry is a cause of tissue damage in many disease states, such as myocardial ischemia. The effect of gossypol, a polyphenolic plant pigment and male contraceptive, on the peroxidation of myocardial membrane phospholipid was studied and quantitatively characterized. As a result of exposure to xanthine oxidase (superoxide)-dependent, iron-promoted Fenton chemistry, cardiac phospholipid was readily peroxidized with defined kinetics. The peroxidation could be blocked by substances which interdict at specific points in the Fenton chemistry: superoxide dismutase, alpha-tocopherol, the iron chelator desferrioxamine, and the xanthine oxidase substrate-analogs allopurinol and oxypurinol. The oxidative-injury system displayed a characteristic antiperoxidant response to each type of inhibitor. Gossypol, at low micromolar concentrations, profoundly altered the rate and extent of myocardial phospholipid peroxidation. Gossypol was ineffective as a xanthine oxidase inhibitor and as a superoxide scavenger at concentrations that abolished myocardial lipid peroxidation. Since metal chelation was an effective means of preventing lipid peroxidation in this system only when the iron therein was completely chelated, the low antiperoxidant IC₅₀ for gossypol, 1.1 microM, relative to the concentration of iron (100 microM) did not support a functionally significant antiperoxidant role for gossypol as an iron chelator. Rather, it appears that, at low micromolar gossypol concentrations which approximate the peak plasma concentrations in humans, the antiperoxidant effects of gossypol against superoxide-mediated, iron-promoted lipid damage rest with the ability of gossypol to intercept lipid radical intermediates as a 'chain-breaking' aromatic phenol.

Protective effect of tea polyphenol on rat myocardial injury induced by isoproterenol

Chinese Traditional and Herbal Drugs (China)(Apr) 1995

The ability of tea polyphenol to protect against myocardial injury induced by isoproterenol was studied in rats. Pretreatment with 10 mg/kg intraperitoneal tea polyphenol 5 days before isoproterenol administration decreased malonyldehyde concentration, and creatine phosphokinase and lactic dehydrogenase activities, and inhibited the extent of myocardial injury similar to the action of propranolol. Plasma renin activity was also decreased.

Effect of the interaction of tannins with coexisting substances. Part 2. reduction of heavy metal ions and solubilization of precipitates

Okuda T; Mori K; Shiota M; Ida K

Yakugaku Zasshi 1982 Aug;102(8):735-42

The precipitate formation in the solution of geraniin

(I), punicalin

(II), tannic acid

(III), or (-)-epigallocatechin gallate

(IV) mixed with that of cadmium, chromium, copper, iron, mercury, manganese, lead or zinc ions at pH 5.4, was investigated. The amount of precipitate decreased with an increase in concentration of I or III but precipitate increased with an elevation of tannin concentration. The precipitates formed were solubilized upon further increase of tannin concentration and when the amount of heavy metal in the supernatant liquor together with the ratio of tannin to heavy metal in the precipitate were increased. Extensive reduction of chromium, ferric, cuprous ions and complex formation occurred in the presence of tannins such as I, II, III and IV. These results indicated that the toxicity of metal ions could be reduced in the presence of tannins and polyphenols.

Free radicals scavenging action and anti-enzyme activities of procyanidines from *Vitis vinifera*. A mechanism for their capillary protective action.

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Arzneimittelforschung 1994 May;44(5):592-601

The scavenging by procyanidines and polyphenol oligomers from *Vitis vinifera* seeds, CAS 85594-37-2) of reactive oxygen species (ROS) involved in the onset (HO degrees) and the maintenance of microvascular injury (lipid radicals R degrees, RO degrees, ROO degrees) has been studied in phosphatidylcholine liposomes (PCL), using two different models of free radical generation: a) iron-promoted and b) ultrasound-induced lipid peroxidation. In a) lipid peroxidation was assessed by determination of thiobarbituric acid-reactive substances (TBARS); in b) by determination of conjugated dienes, formation of breakdown carbonyl products (as 2,4-dinitrophenylhydrazones) and loss of native phosphatidylcholine. In the iron-promoted (Fenton-driven) model, procyanidines had a remarkable, dose-dependent antilipoperoxidant activity ($IC_{50} = 2.5 \text{ } \mu\text{mol/l}$), more than one order of magnitude greater than that of the monomeric unit catechin ($IC_{50} = 50 \text{ } \mu\text{mol/l}$), activity which is due, at least in part, to their metal-chelating properties. In the more specific model b), which discriminates between the initiator (hydroxyl radical from water sonolysis) and the propagator species of lipid peroxidation (the peroxy radical, from autooxidation of C-centered radicals), procyanidines are highly effective in preventing conjugated diene formation in both the induction ($IC_{50} = 0.1 \text{ } \mu\text{mol/l}$) and propagation ($IC_{50} = 0.05 \text{ } \mu\text{mol/l}$) phases (the scavenging effect of alpha-tocopherol was weaker, with IC_{50} of 1.5 and 1.25 $\mu\text{mol/l}$). In addition, procyanidines at 0.5 $\mu\text{mol/l}$ markedly delayed the onset of the breakdown phase (48 h), totally inhibiting during this time the formation of degradation products (the lag-time induced by alpha-tocopherol was only of 24 h at 10 $\mu\text{mol/l}$ concentration). The HO degrees entrapping capacity of these compounds was further confirmed by UV studies and by electron spin resonance (ESR) spectroscopy, using DMPO as spin trapper: procyanidines markedly reduced, in a dose-dependent fashion, the signal intensity of the DMPO-OH radical spin adduct (100% inhibition at 40 $\mu\text{mol/l}$). The results of the second part of this study show that procyanidines, in addition to free radical scavenging action, strongly and non-competitively, inhibit xanthine oxidase activity, the enzyme which triggers the oxy radical cascade ($IC_{50} = 2.4 \text{ } \mu\text{mol/l}$). In addition procyanidines non-competitively inhibit the activities of the proteolytic enzymes collagenase ($IC_{50} = 38 \text{ } \mu\text{mol/l}$) and elastase ($IC_{50} = 4.24 \text{ } \mu\text{mol/l}$) and of the glycosidases hyaluronidase and beta-glucuronidase ($IC_{50} = 80 \text{ } \mu\text{mol/l}$ and 1.1 $\mu\text{mol/l}$), involved in the turnover of the main structural components of the extravascular matrix collagen, elastin and hyaluronic acid.

The inhibitory action of chlorogenic acid on the intestinal iron absorption in rats.

Gutnisky A, Rizzo N, Castro ME, Garbossa G
Centro de Estudios Farmacologicos y Botanicos, Buenos Aires, Argentina.
Acta Physiol Pharmacol Ther Latinoam 1992;42(3):139-46

The polyphenols are part of the composition of many foods, it is known the inhibitory effect of tea and coffee through the tannins on iron intestinal absorption; the "yerba mate" (*Ilex Paraguarensis*) is a beverage widely used in South America, that has a high content of a polyphenol named chlorogenic acid. The present work shows the effect of this substance in nonhem iron absorption. An intestinal loop, was made in rats, to form a closed cavity in a small section of intestine tying it from the pilorus to a distance of six cm. In this closed cavity a solution of ^{59}Fe was injected with different doses of chlorogenic acid; it was living 20, 40 and 120 minutes into the loop, and after this different times, the blood, spleen, liver, femur and intestine were removed to measure the ^{59}Fe uptake to be compared with the control group. The results gave an intense inhibitory effect on the intestinal iron absorption with doses of 0.58 and 1.7 mM per rat of chlorogenic acid at the different times studied.

Inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis by compounds derived from cruciferous vegetables and green tea.

Chung FL, Morse MA, Eklind KI, Xu Y
Division of Chemical Carcinogenesis American Health Foundation, Valhalla, New York 10595.
Ann N Y Acad Sci 1993 May 28;686:186-201; discussion 201-2

We have shown that PEITC and I3C, both of cruciferous origin, inhibited lung tumor formation induced by the tobacco-specific nitrosamine NNK. The inhibition by PEITC is due largely to its inhibitory effect on the enzymes of NNK metabolism, whereas; the inhibition by I3C may be attributed to its ability to induce hepatic enzyme activity of NNK metabolism, which resulted in decreased availability of NNK to the lung. One NNK-induced lung umorigenesis, probably due to its antioxidant property. These studies provide for the first time evidence for the involvement of free radicals in nitrosamine tumorigenesis. The mechanism by which free radicals are generated by NNK treatment is not yet known. The reduced levels of oxidative lesions in lung as a result of EGCG treatment may be related to its ability to reduce reactive oxygen species and/or to chelate iron ion resulting in a decreased production of hydroxyl radicals. Overall, these studies have identified ingredients in cruciferous vegetables and green tea that are inhibitory against lung tumorigenesis induced by NNK in rodents.

Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption.

The effects of maize-bran phytate and of a polyphenol (tannic acid) on iron absorption from a white-bread meal were tested in 199 subjects. The phytate content was varied by adding different concentrations of phytate-free and ordinary maize bran. Iron absorption decreased progressively when maize bran containing increasing amounts of phytate phosphorous (phytate P) (from 10 to 58 mg) was given. The inhibitory effect was overcome by 30 mg ascorbic acid. The inhibitory effects of tannic acid (from 12 to 55 mg) were also dose dependent. Studies suggested that greater than or equal to 50 mg ascorbic acid would be required to overcome the inhibitory effects on iron absorption of any meal containing greater than 100 mg tannic acid. Our findings indicate that it may be possible to predict the bioavailability of iron in a diet if due account is taken of the relative content in the diet of the major promoters and inhibitors of iron absorption.

Phytic acid. A natural antioxidant.

Graf E, Empson KL, Eaton JW
J Biol Chem 1987 Aug 25;262(24):11647-50

The catalysis by iron of radical formation and subsequent oxidative damage has been well documented. Although many iron-chelating agents potentiate reactive oxygen formation and lipid peroxidation, phytic acid (abundant in edible legumes, cereals, and seeds) forms an iron chelate which greatly accelerates Fe²⁺-mediated oxygen reduction yet blocks iron-driven hydroxyl radical generation and suppresses lipid peroxidation. Furthermore, high concentrations of phytic acid prevent browning and putrefaction of various fruits and vegetables by inhibiting polyphenol oxidase. These observations indicate an important antioxidant function for phytate in seeds during dormancy and suggest that phytate may be a substitute for presently employed preservatives, many of which pose potential health hazards.

[Effect of polyphenols of coffee pulp on iron absorption]

de Roza MP, Velez J, Garcia LA
Arch Latinoam Nutr 1985 Jun;35(2):287-96

The effect of the polyphenols of coffee pulp on iron absorption was studied using the method of ligated segments in rats. Optimal conditions to measure iron absorption, were determined using as criteria the concentration of Fe⁵⁹ and the time that produced the highest value of blood radioactivity. A concentration of 0.4 uCi/dose of Fe⁵⁹ and a 3-hr period were chosen to measure iron absorption. Experimental groups were formed assigning six rats randomly to each group. Each group was injected with a solution of ⁵⁹Fe and either with the standard polyphenol solution or with the coffee pulp extract, except the control group which was injected with the Fe⁵⁹ solution only. The effect of two polyphenol concentrations was also studied. Iron uptake from the duodenum was found to be the best indicator of iron absorption when compared to the sum of iron uptake by the tissues (blood, liver, spleen, kidneys, heart and carcass). Therefore, this indicator was used to interpret the results obtained. Catechin, tannic acid and the coffee pulp extract decreased significantly iron absorption when compared with the control group. The level of polyphenols used in these experiments is similar to the amounts consumed by animals fed coffee pulp at a 10% level. Therefore, we can conclude that the antinutritional effect of coffee pulp polyphenols may be partially due to their capacity to bind iron.

Factors affecting the absorption of iron from cereals.

Gillooly M, Bothwell TH, Charlton RW, Torrance JD, Bezwoda WR, MacPhail AP, Derman DP, Novelli L, Morrall P, Mayet F
Br J Nutr 1984 Jan;51(1):37-46

Non-haem-iron absorption from a variety of cereal and fibre meals was measured in parous Indian women, using the erythrocyte utilization of radioactive Fe method. The present study was undertaken to establish whether alteration of the phytate and polyphenol contents of sorghum (*Sorghum vulgare*) affected Fe absorption from sorghum meals, and to assess the influence of fibre on Fe absorption. Removing the outer layers of sorghum grain by pearling reduced the polyphenol and phytate contents by 96 and 92% respectively. This treatment significantly increased the geometric mean Fe absorption from 0.017 to 0.035 (t 3.9, P less than 0.005). The geometric mean Fe absorption from a sorghum cultivar that lacked polyphenols (albino sorghum) was 0.043, which was significantly greater than the 0.019 absorbed from bird-proof sorghum, a cultivar with a high polyphenol content (t 2.83, P less than 0.05). Fe was less well absorbed from the phytate-rich pearlings of the albino sorghum than from the pearled albino

sorghum (0.015 v. 0.035 (t 8.4, P less than 0.0005]. Addition of sodium phytate to a highly Fe-bioavailable broccoli (*Brassica oleracea*) meal reduced Fe absorption from 0.185 to 0.037. The geometric mean Fe absorption from malted sorghum porridge was 0.024 when 9.5 mg ascorbic acid were added and 0.094 when the ascorbic acid was increased to 50 mg (t 3.33, P less than 0.005). This enhancing effect of 50 mg ascorbic acid was significantly depressed to 0.04 by tea (t 38.1, P less than 0.0005).

The effect of red and white wines on nonheme-iron absorption in humans.

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Am J Clin Nutr 1995 Apr;61(4):800-4

The effect of the phenolic compounds in wine was examined in this study by performing radioiron-absorption measurements from extrinsically labeled test meals in 33 human subjects. In four separate studies we observed that absorption was 2- to 3-fold higher from white wine containing a low concentration of polyphenols than from two red wines containing a 10-fold higher concentration of polyphenols. The interaction between the polyphenols and alcohol in wine was evaluated by reducing the alcohol content of the wines by approximately 90%. When the alcohol concentration was reduced, there was a significant 28% decrease in nonheme-iron absorption with red wine but no effect with white wine. The inhibitory effect of red wines with reduced alcohol content was about twofold greater when they were consumed with a small bread roll than when taken without food. Our findings indicate that the inhibitory effect of phenolic compounds in red wine is unlikely to affect iron balance significantly.

Prevention of iron deficiency.

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Baillieres Clin Haematol 1994 Dec;7(4):805-14

This chapter discusses different methods to prevent iron deficiency--to reduce iron losses (e.g. reducing menstrual iron losses by using a contraceptive pill or combating of hookworm infestation) or to increase iron absorption. Iron absorption can be increased

(1) by modifying the composition of meals--increasing the content of dietary factors enhancing iron absorption (e.g. meat and ascorbic acid) or reducing the content of factors inhibiting iron absorption such as phytate and iron-binding phenolic compounds,

(2) by increasing the iron content of the diet by fortification with iron, or by

(3) supplementation with iron tablets. Several factors to consider in the choice of strategy are discussed such as the importance of the bioavailability of the diet for the efficacy of iron fortification, the choice of vehicle for iron fortification that is compatible with the iron compound used, the feasibility to increase the bioavailability of the dietary iron by modification of the composition of the diet and the short time available in pregnancy to ensure a sufficient supply of the extra iron needed limiting the effective measures available to supplementation with iron tablets.

Iron absorption and phenolic compounds: importance of different phenolic structures.

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The phenolic compounds (phenolic monomers, polyphenols, tannins) are considered to interfere with iron absorption by complex formation with iron in the gastro-intestinal lumen, making the iron less available for absorption. Very little is known about the extent to which different types of phenolic compounds of different size and chemical structure inhibit iron absorption. The relationship between iron absorption and the amount and type of phenolic compounds was studied by the extrinsic tag method. The aims of the studies were as follows:

(i) To study the effect of small phenolic compounds with different hydroxylation patterns (gallic acid, catechin, chlorogenic acid) on iron absorption,

(ii) To study the effect of different amounts of a hydrolysable tannin containing ten gallic acid residues (tannic acid) on iron absorption.

(iii) To study the degree of inhibition of iron absorption by some foods and beverages (oregano, spinach, coffee and tea) in relation

to their respective content of iron-binding phenolic groups, measured by a newly developed method. The inhibition of iron absorption by tannic acid was strongly dose-related. The smallest amount (5 mg) inhibited absorption by 20 per cent, 25 mg by 67 per cent and 100 mg by 88 per cent. Gallic acid inhibited iron absorption to the same extent as tannic acid, per mol galloyl groups, whereas no inhibition was observed when catechin was added to the test meal. Chlorogenic acid inhibited iron absorption to a lesser extent. Oregano and tea inhibited iron absorption in proportion to their respective content of galloyl groups, whereas the inhibitory effect of spinach was less marked. The inhibiting effect of coffee was explained mainly by its content of galloyl groups, but also by some other factor, probably chlorogenic acid. It is concluded that the content of iron-binding galloyl groups might be a major determinant of the inhibitory effect of phenolic compounds on iron absorption from the diet, whereas the phenolic catechol groups seem to be of minor importance. The results further suggest that the group of condensed tannins do not interfere with iron absorption.

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