Fructose intolerance: diet and inheritance

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Hereditary Fructose Intolerance (HFI; first recognized as a clinical entity by Chambers & Pratt, (1956)) provides a vivid example of interactions between changing dietary factors and inheritance in the development of human disease. Although the cultivation of sugar has its roots in antiquity, its importance as a foodstuff has developed only over the last 300 or so years with the rise of sugar-cane cultivation and the cultivation and extraction of sugar beet. For these reasons, HFI, which affects about one in 20 000 of the population, is a disease largely induced by the recent introduction of sugar as a major constituent of the diet.

HFI is an autosomal recessive disorder which is caused by a deficiency of fructose-1-phosphate aldolase (*EC* 4.1.2.13; aldolase B) in the liver, intestine and kidney (Gitzelmann *et al.* 1989). Exposure to fructose, sucrose and sorbitol induces abdominal pain and profound metabolic disturbances, including hypoglycaemia, that may be fatal. However, the response to dietary exclusion treatment is rapid and, when harmful foods are avoided, patients with HFI recover completely and remain in good health. After a stormy period following weaning and the introduction of fruits and sugars into the diet, an aversion to sweet-tasting foods (as well as fruits) usually develops. This allows affected children and adults to escape the worst effects of the disorder. It is thus likely that selection against the condition was minimal before the introduction of fructose- and sucrose-containing foods into the diet.

The clinical features of HFI include vomiting, diarrhoea and abdominal pain following the ingestion of fructose-containing foods. Fructose or sucrose induces disturbed consciousness and, on occasion, convulsions due to hypoglycaemia. There may be a failure to gain weight and a syndrome of 'failure to thrive'. At this stage, survival of the infant is dependent on the identification by the mother of feeds that provoke the worst effects. Continued ingestion of sugar leads to severe liver disease with cirrhosis, a terminal haemorrhagic diathesis, metabolic acidosis with structural disease of the kidney, and growth retardation (Odièvre *et al.* 1978). Should the infant survive to childhood, the consumption of fructose and sucrose from all sources is usually reduced to 1 or 2 g only and there is a partial remission of the symptomatology. However, given the ubiquity of fructose, patients may develop a syndrome of chronic fructose intoxication with retardation of growth, bone disease and chronic liver disease, and this may also be punctuated by episodes of hypoglycaemia.

ENZYME DEFECT IN HFI

It was shown by Hers in 1961 (Hers & Joassin, 1961) that the enzymic defect in HFI was a functional deficiency of fructose-1-phosphate aldolase. Aldolase B, in which fructose-1-phosphate aldolase activity is invested in fructose-metabolizing tissues, is responsible for the cleavage of fructose-1-phosphate into D-glyceraldehyde and dihydroxyacetone

phosphate. These sugars enter the intermediary pools of carbohydrate metabolism and may be re-condensed to form fructose-1,6-diphosphate that enters the pathway for glycogen formation, gluconeogenesis, or glycolysis. In liver biopsy samples from patients with HFI there is a catalytic deficiency of aldolase B such that fructose-1-phosphate activity is reduced to less that 5% of normal and there is a partial deficiency of fructose-1,6-diphosphate aldolase activity. This results in activity ratios of fructose-1,6-diphosphate aldolase:fructose-1-phosphate aldolase of > 5:1 in HFI compared with that of normal liver, where the ratio is less than 2. The residual aldolase activities in affected tissues are attributable to the constitutive aldolases (A and C) which maintain gluconeogenic and glycolytic fluxes in the absence of fructose challenge.

METABOLIC DISTURBANCE IN HFI

The metabolic disturbance that is induced by fructose is complex (Froesch *et al.* 1963; Cox, 1988; Gitzelmann *et al.* 1989). Phosphorylation of ingested fructose occurs in the liver and intestine and is rapid. In the absence of aldolase B activity, fructose-1-phosphate accumulates in the tissues and there is a sequestration of inorganic phosphate. Studies employing ^{31}P magnetic-resonance spectroscopy have shown in man that up to 80% of liver inorganic phosphate is removed by the infusion of small quantities of fructose. The accumulation of fructose-1-phosphate and the depletion of inorganic P within the fructose-metabolizing tissues initiates the metabolic disturbance. Under these circumstances, there is competitive inhibition of aldolase A activity in the direction of triose condensation, leading to an inhibition of gluconeogenesis. There is, moreover, a rapid inhibition of phosphorylase B (*EC* 2.4.1.1) activity and this inhibits glycogenolysis. Thus, during hypoglycaemia induced by fructose, administration of glucagon, dihydroxyacetone or glycerol does not restore the blood glucose concentration. The activity of phosphoglucomutase (*EC* 5.4.2.2) is unimpaired, however, and galactose infusions relieve the hypoglycaemia promptly. Among the other abnormalities induced by fructose, is the activation of adenosine deaminase (*EC* 3.5.4.4) so that degradation of purine nucleotides is stimulated. Hence, fructose induces marked hyperuricaemia. Additional secondary effects of fructose ingestion include fructosaemia and fructosuria which result from the competitive inhibition of ketohexokinase (*EC* 2.7.1.3) by fructose-1-phosphate and a metabolic acidosis consequent on the accumulation of lactate. Disturbances of proximal renal tubular function lead to the wasting of bicarbonate ions and a Fanconi-like syndrome with renal tubular acidosis.

CLINICAL GENETICS

Clinical studies of the genetic transmission of HFI indicate that it is inherited as a recessive character, although reports of consanguinity in the parents of affected children are rare. Several families have been reported where there has been vertical transmission of disease from parent to offspring and it has been shown that this occurs when there is a mating of an affected homozygote with an asymptomatic carrier of the condition. The frequency of such pedigrees indicates that HFI genes are relatively prevalent in the population at large. Hitherto, no reproducible changes in biochemical measurements have been identified in obligate heterozygotes, even when they are challenged with fructose. Although some abnormalities of hepatic metabolism of fructose monoesters

have been identified by ^{31}P magnetic-resonance spectroscopy after fructose loading, no simple method has been devised hitherto for identifying asymptomatic disease carriers.

MOLECULAR GENETICS

The molecular basis of aldolase B deficiency has been the subject of intensive investigations and has been greatly helped by the elucidation of the gene sequence (Tolan & Penhoet, 1986). Early experiments using isoenzyme-specific antibodies demonstrated the presence of catalytically-impaired aldolase B in biopsy material taken from patients with the disorder (Nordmann *et al.* 1968; Grégori *et al.* 1982; Cox *et al.* 1983). Recently a prevalent mutation in the gene for aldolase B was identified in a British patient who had fathered three children with the disease. The mutation, which is a G->C transversion, results in the substitution of a proline residue for the amino acid alanine at position 149 of the protein (Cross *et al.* 1988). This lesion is, therefore, designated A149P. It was identified in the first British patient who was homozygous for it as well as his three offspring who were affected by HFI. A149P is prevalent in patients with HFI from the UK.

Subsequently, six further aldolase B lesions have been identified. Three of these are point mutations affecting the coding region. One, designated A174D, is another widespread missense mutation which is caused by a C->A transversion and which causes an invariant alanine in position 174 of the protein to be replaced by an aspartic acid residue (Cross *et al.* 1990). Another mutation, N344K, results in the substitution of a lysine residue for the normal asparagine at position 334 of the protein and appears to be prevalent in patients from Eastern Europe. A number of sporadic aldolase B variants appear to be more restricted in distribution and result from deletions ranging from one base pair (L288ΔC) to 1·65 kb in the aldolase gene and which all grossly disturb the synthesis of active enzyme.

The missense mutations are particularly important since their study at the functional level will give an indication of the structural requirements in regions of the enzyme that are essential for its normal catalytic properties. In preliminary work, it has been possible to express wild-type human aldolase B by use of an expression plasmid vector in *Escherichia coli*. This enzyme has catalytic properties in relation to Michaelis constant and substrate-activity ratios that are indistinguishable from normal human liver aldolase B. When the mutant enzyme A149P was synthesized after site-directed mutagenesis in the same expression system, the catalytic activities were found to be below 0·5% of normal, indicating that the mutation was sufficient to account for the enzymic defect.

GENETIC DIAGNOSIS OF HFI

It is now possible to detect these mutations in aldolase B in patients with HFI by a rapid screening procedure. The automated synthesis of oligonucleotides and the introduction of the polymerase chain reaction (PCR) has been of immediate applicability in the analysis of genetic lesions. By hybridizing labelled mutation-specific probes to sequences amplified by the PCR, lesions in the gene can be detected using minute quantities of input DNA that can be obtained non-invasively and transported easily for analysis. This general method has been utilized to study many patients with HFI from all over Europe and it has been possible to determine the frequency of different alleles of aldolase B in

patients that originate from diverse population groups. It is remarkable that the most prevalent alleles (A149P and A174D) may be conveniently detected by the use of wild-type and mutation-specific oligonucleotides successively to probe amplified sequences from the same exon of aldolase (Cross *et al.* 1990). It is now clear that, given the remarkable prevalence of a few point mutations in mutant alleles of aldolase B, the vast majority of patients with HFI will be amenable to direct genetic diagnosis without the need for invasive procedures such as visceral biopsy or the standard intravenous fructose tolerance test that has been in clinical use until now. The procedure may be extended to the identification of heterozygous carriers of the disorder in affected pedigrees and represents a distinct improvement on currently available procedures. Genetic diagnosis has already been carried out successfully in a number of patients with previously undiagnosed symptoms which were suggestive of HFI and who had requested diagnostic tests on their own initiative. On several occasions it has proved possible to obtain decisive information using DNA obtained from epithelial cells present in mouthwash samples. All these individuals have been greatly helped by formal diagnosis and institution of a strict fructose-, sucrose-, and sorbitol-free diet.

POPULATION GENETICS

The remarkable distribution of a few missense mutations in the European population suggest that these common mutations have spread throughout the geographical regions by genetic drift and probably arose as a single event in a common ancestor. Heterozygotes manifest no apparent symptoms or advantage but it remains possible that the striking absence of dental caries in most adults in HFI may have conferred a selective advantage that was not counter-balanced by dietary deficiencies of vitamin C or folic acid. The most probable explanation for the extraordinary prevalence of aldolase B mutations (heterozygote frequency estimated at one in seventy of the population) is that sugar has only recently become the major constitutent of the diet. In England in 1700 the estimated daily consumption of sugar was 4·5 g per capita and, before that, the major dietary source of fructose was in honey, a luxury item. By 1800, in England, 37 g sucrose per capita was consumed daily and in 1968 this had risen to 140 g per capita daily. World sugar production has increased to the extent of 60 g per capita daily and this now provides a measure of the ubiquity of this adverse dietary factor for patients harbouring hereditary deficiencies of aldolase B.

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Printed in Great Britain