



Fructose-associated hepatotoxicity indexed by the lactate dehydrogenase isoenzyme LDH-5

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ABSTRACT

Modern diets have become increasingly rich in fructose, for example through the addition of high-fructose corn syrup to many foods and drinks. It has been suggested that this might lead to hepatotoxicity, including the development of non-alcoholic fatty liver disease. After entering hepatocytes via insulin-independent glucose transporter 2 transmembrane carrier proteins, fructose is phosphorylated to fructose-1-phosphate in a reaction catalysed by fructokinase (ketoheokinase). In turn, fructose-1-phosphate is hydrolysed by aldolase B to glyceraldehydes. Glyceraldehydes may enter gluconeogenesis via fructose-1,6-bisphosphate and fructose-6-phosphate; glyceraldehydes may also enter glycogenolysis via pyruvate. The last pathway involves conversion of pyruvate to acetyl-CoA. Alternatively, pyruvate may be converted, via the action of the hepatic lactate dehydrogenase isoenzyme LDH-5, into lactate. In liver damage, the LDH-5 isoenzyme becomes elevated, predominantly in serum/plasma. We therefore hypothesised that if dietary fructose is associated with hepatotoxicity, there should be a positive correlation between erythrocyte fructose-6-phosphate and plasma LDH-5. This hypothesis was tested by assaying venous blood samples taken from 39 patients at rest, three hours after eating. Quantitative Fourier transform infrared spectrometry following gel electrophoresis was used to assay erythrocyte fructose-6-phosphate levels. Similarly, plasma LDH-5 concentrations were spectrophotometrically analysed, using the pyruvate-lactate reaction, following electrophoretic separation of the LDH isoenzymes. A significant positive correlation was found between the two variables ($r = 0.44$, $p = 0.0047$). This result, which supports our hypothesis, is evidence in favour of the possibility that dietary fructose is associated with hepatotoxicity. In addition to being a marker of hepatic damage, LDH-5 may play a more direct epigenetic role in causing liver damage; acute hepatic injury is associated with nuclear translocation of LDH, causing the production of lactate from pyruvate in the nucleus; in turn, the lactate inhibits histone deacetylase and is associated with upregulation of genes associated with the damage response, leading to cell death.

Introduction and background

Modern diets have become increasingly rich in fructose, for example through the addition of high-fructose corn syrup (HFCS) to many foods and drinks [1–3]. It has been suggested that this might lead to hepatotoxicity, including the development of non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH); mice fed on a combination of HFCS and trans fats, known as the American lifestyle-induced obesity syndrome (ALIOS) model, are particularly prone to develop obesity and severe NAFLD with hepatic necroinflammatory changes [4]. Indeed, human NAFLD driven by added fructose has been termed a public health crisis [5].

After it enters hepatocytes via insulin-independent glucose transporter 2 (GLUT2) transmembrane carrier proteins [6], fructose is phosphorylated to fructose-1-phosphate in a reaction catalysed by hepatic fructokinase (or ketoheokinase) [7]. In turn, the fructose-1-phosphate is hydrolysed by aldolase B (fructose-1-phosphate aldolase) to glyceraldehydes [8,9]. Glyceraldehydes may enter gluconeogenesis via fructose-1,6-bisphosphate and fructose-6-phosphate; glyceraldehydes may also enter glycogenolysis via the alpha-keto acid

pyruvate; the latter pathway involves the conversion of pyruvate to acetyl coenzyme A (acetyl-CoA) [10]. An alternative pathway open to pyruvate is its conversion, via the action of the hepatic lactate dehydrogenase (LDH) isoenzyme LDH-5, into lactate [11–13].

The LDH oxidoreductase isoenzymes, or isozymes, are tetramers of ‘muscle’ (M) and ‘heart’ (H) active subunits [11,13]. LDH-5 is the tetramer M₄ and is contained in the cytoplasm of cells of essentially all tissues; the main sources of serum LDH-5 activity are liver damage, skeletal muscle damage, for example following strenuous exercise, and erythrocytic LDH-5 following haemolysis [14,15]. Thus, if blood samples are taken from subjects who do not suffer from primary skeletal muscle disease and who are at rest, and if the samples are not haemolysed, then elevated plasma or serum LDH-5 activity should index liver damage [14,15].

The hypothesis

Based on the above considerations we hypothesised that, if dietary fructose is associated with hepatotoxicity, there should be a positive correlation between erythrocyte fructose-6-phosphate and plasma LDH-

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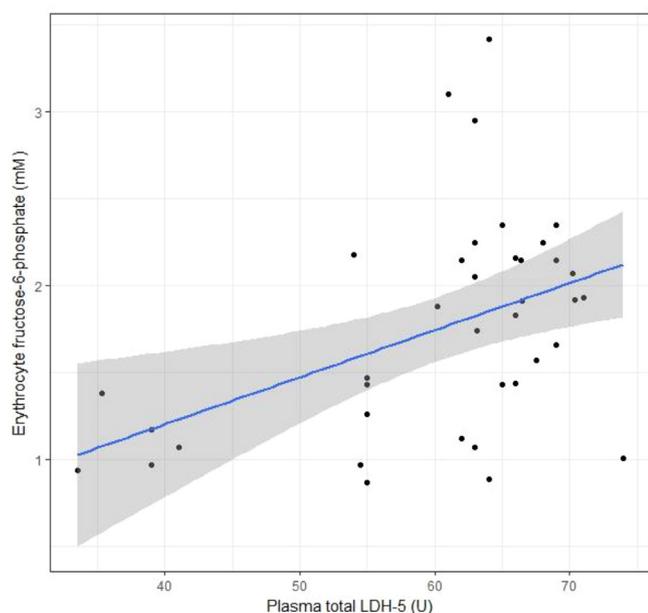


Fig. 1. Erythrocyte fructose-6-phosphate levels plotted against LDH-5 plasma levels with the linear regression line and its 95% confidence intervals.

5. The aim of this study was to test this hypothesis in subjects who did not suffer from primary skeletal muscle disease and from whom non-haemolysed blood samples were to be taken at rest.

Evaluation of the hypothesis

This hypothesis was tested by assaying venous blood samples taken from 39 patients at rest, three hours after eating. None of the patients suffered from primary skeletal muscle disease and care was taken to ensure that none of these blood samples was haemolysed. Sixteen of the patients were male and the remaining patients were female; their mean age was 42 years. They attended an environmental medicine clinic. This audit was approved by the AONMREC and the study was carried out in accordance with the Declaration of Helsinki.

Quantitative Fourier transform infrared spectrometry following gel electrophoresis was used to assay erythrocyte fructose-6-phosphate levels. Similarly, plasma LDH-5 concentrations were spectrophotometrically analysed, using the pyruvate-lactate reaction, following electrophoretic separation of the LDH isoenzymes. The assays were carried out, following separation of blood fractions using centrifugation, by Acumen laboratories (Tiverton), independently of the investigators.

Version 3.4.2 of R was used for data analysis, running on an x86_64-w64-mingw32/x64 (64-bit) platform [16]. Linear regression was carried out between plasma LDH-5 and erythrocyte fructose-6-phosphate after checking normality by analysing diagnostic plots, including residuals versus fitted values, (standardised residuals)^{1/2} versus fitted values, standardised residuals versus leverage and a normal quantile-quantile probability plot.

A significant positive correlation was found between the erythrocyte fructose-6-phosphate and plasma LDH-5 levels ($r = 0.4438$, $t = 3.0123$, $df = 37$, $p = 0.0047$). A plot of these data is shown in Fig. 1, which also shows the corresponding linear regression line and its 95 per cent

confidence intervals.

Discussion

This result, which supports our hypothesis, is evidence in favour of the possibility that dietary fructose is associated with hepatotoxicity. In addition to being a marker of hepatic damage, LDH-5 may play a more direct role in causing cell damage in the liver. In an elegant set of murine experiments, Ferriero and colleagues have recently shown that acute hepatic injury is associated with nuclear translocation of LDH and pyruvate dehydrogenase complex, causing the production of lactate from pyruvate in the nucleus as well as increased nuclear acetyl-CoA; in turn, hyper-acetylation of histone H3 takes place, which is associated with upregulation of genes associated with the damage response, leading to cell death [17].

In sum, our pilot study suggests that increased dietary fructose is associated with epigenetic changes which lead to cellular death in the liver.

Conflicts of interest

None.

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