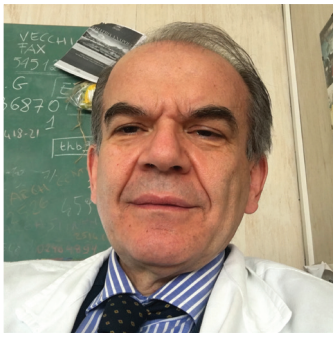
DIET AND LIVER DISEASES



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- **Hereditary Fructose intolerance: impact of daily fructose traces intake on liver health**
- **Wilson's disease: does the low copper diet help the patient treated pharmacologically?**

Background

Hereditary fructose intolerance (HFI) is a rare genetic disorder of fructose metabolism due to aldolase B enzyme deficiency. Treatment consists on fructose, sorbitol and sucrose free diet. Total exclusion of these sugars is not easily feasible because of small hidden fructose amounts contained in many foods. It is also not clear whether small amounts of fructose can be tolerated and if a threshold exists, above which liver damage occurs. Therefore, possible correlations between daily fructose traces intake and liver injury biomarkers on a long-term period, in a cohort of young patients affected by HFI have been investigated by our group. Correlations among fructose intake, serum alanine aminotransferase (ALT) level, carbohydrate-deficient transferrin (CDT) percentage, liver ultrasonography, genotype have been analyzed. In brief, we are interested to clarify the general clinical conditions, the auxological parameters and the psychomotor status after a long period of fructose, sorbitol and sucrose free diet and the role of fructose traces on liver health in HFI patients.

Wilson's disease (WD) is an autosomal-recessive human copper (Cu) storage disorder caused by mu-tations in the ATP7B gene. Clinical presentation can vary widely,

but the key features of WD are liver and neurological disease and neuropsychiatric disturbances. The ATP7B gene encodes copper-translocating ATPase expressed primarily in the liver. The exact mechanism of copper hepatotoxicity and brain injury remains not totally clarified. The overall therapeutic aim for WD is the generation of a negative copper balance. Conventional medical therapies, which must be performed throughout life, in-clude chelators (penicillamine or trientine) and zinc. Dietary copper is usually recommended in WD patients in the first phases of pharmacological treatment. Therefore avoiding copper-rich food (shell-fish, nuts, chocolate, mushrooms, and organ meats) is advised until remission of symptoms and bio-chemical abnormalities. This suggestion is mainly aimed at patients on chelator therapy, while dietary restriction of copper should be less thrust for patients treated with zinc. Our goal is to clarify the impact of daily copper dietary content on the liver balance of WD patients both those treated with chelators and those treated with zinc.

Main achievements

As for Hereditary Fructose Intolerance (HFI), we have recently evaluated a series of 48 HFI patients followed at Units of Metabolic diseases (Prof Parenti) and Liver diseases of Department of Translational Medical Science, Section of Pediatrics, University of Naples Federico II. All patients followed the prescribed diet without sucrose, fructose and sorbitol. Their fructose intake was 169±145.4 mg/day confirming the difficulty in totally excluding non-tolerated sugars. Even if they ingested these traces of fructose, at the end of a follow-up of 10.3±5.6 years, these patients showed good general conditions, normal growth and psychomotor development. Interestingly, about all of them had hepatic steatosis at sonographic evaluation and a third persistent mild hypertransaminasemia. Fructose intake did not correlate with aminotransferases levels nor with steatosis severity, whereas it correlated with disialotrasferrin percentage and tetrasialotransferrin/disialotransferrin ratio. As for mutational analysis, p.A150P mutation homozygotes patients had lower ALT values at diagnosis than p.A175D variant homozygotes cases. Therefore, the genotype rather than the daily fructose intake seemed to play a role in hypertransaminasemia. Furthermore, our studies have clarified that hepatic steatosis, a hallmark feature of HFI, and a mild hypertransaminasemia can be found also in patients adherent to fructose, sorbitol and sucrose (FSS) free diet.

As for Wilson's disease, our group has coordinated within the framework of the Italian Society of Pediatric Gastroenterology Hepatology and Nutrition (SIGENP) multicentre studies on the behavior of transaminases in pediatric patients with Wilson's disease (WD) under therapy and genotype-phenotype correlation. The first study showed that about one third of children with WD continues to have mild-to-moderate

persistent hypertransaminasemia despite an adequate pharmacological treatment with penicillamine or zinc (Hepatology 2004; 39 (4):1173-4). This is an interesting result because it avoids that this subgroup of patients is subjected to expensive diagnostic investigations. In the study on the genotype-phenotype correlation (J Hepatol 2009; 50:555-61), it has been demonstrated that the type of mutation has an effect on the clinical and laboratory expression of the disease (non-sense and frameshift mutations are associated with more severe phenotypes than missense ones) and also on the response to therapy. In addition, for the first time, in this study a linear correlation between the values of 24-hour cupriuria levels and age of patients at diagnosis was demonstrated. Moreover considering that the diagnosis of WD is challenging especially in children, all WD diagnostic criteria were analyzed in a large cohort of Italian children, concluding that urinary copper excretion greater than 40 μg/24 hours is suggestive of WD in asymptomatic children, whereas the penicillamine challenge test does not have a diagnostic role in this subset of patients (Hepatology 2010;52:1948-56).

Since the treatment of WD patients with mild liver disease was not clearly defined, our group has evaluated long-term outcomes of three treatment regimens (D-penicillamine, zinc or both) in patients diagnosed in childhood (Orphanet J Rare Dis 2014;9:41).

Our group has also participated in multicentre studies regarding the genetic aspects and pathogenesis of WD. An early study (Test Genet 2007; 11 (3):328-32) evaluated the spectrum of mutations in the ATP7B gene in 134 Italian families of patients affected by WD. This study has helped to improve the strategy of performing molecular analysis for diagnostic purposes. In another collaborative multicentre study (Genet Test Mol Biomarkers 2009; 13 (2):185-91), the role of some splicing mutations at the RNA level has been clarified. In this study we reported the RNA molecular characterization of three consensus splice-site mutations identified by DNA analysis in WD patients. Moreover these studies, helped to better establish the molecular mechanisms producing WD (Gene. 2015 Sep 15;569(2):276-9). In the context of pathogenetic mechanisms of WD, it must be considered the study on the role of MURR1 in the pathogenesis of WD as well as in other WD-like disorders of hepatic copper metabolism of unknown origin. The results of this study showed that the MURR1 gene and its protein product are unlikely to play a primary role in the pathogenesis of WD (J Gastroenterol 2006; 41 (6):582-7). Our group in recent years is strongly committed not only to the study of pathogenesis but also to the search for innovative therapies that are additional to conventional treatments. In this context, we are interested:

- To determine new molecular mechanisms involved in the pathogenesis of WD
- To identify molecular targets that can be used for therapeutic approaches in WD
- To identify pharmacologically active components, with the subsequent potential development of new drugs to treat the WD

- To test the best components directly in vitro on hepatocyte derived from fibroblasts of WD patients
- To test the best components in vivo directly in animal model (C. elegans) to validate a new drug in WD patients.

Future perspectives

As for HFI, our study aimed at assessing the impact of fructose traces on the liver health of patients will allow to better manage the diet of these patients. Considering that liver damage seems more related to the type of mutation, a personalized dietary approach will be possible. The knowledge furthermore that a liver disease is present in a good part of fructosemic patients will avoid useless and costly investigations, especially in those adherents to the diet. Since we found a linear correlation between fructose intake and serum disialotrasferrin percentage and tetrasialotransferrin/disialotransferrin ratio, serum carbohydrate-deficient transferrin (CDT) determination can be considered a good tool to monitor fructose, sucrose and sorbitol intake in HFI patients. We could suppose that normal CDT profile is the desirable therapeutic target in HFI patients. Furthermore, CDT profile could be useful to suggest maximum fructose daily intake tolerability of each HFI patient for a personalized diet therapy.

As for Wilson's disease, our studies on daily copper intake in WD patients treated or with chelators or zinc will allow us to clarify whether the diet can play an additional role in the treatment of Wilson's disease. This evaluation will be performed in groups of patients different for age, genotype, clinical aspects and type of pharmacological therapy.

Publications

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