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Blood redox status is associated with the likelihood of nonalcoholic fatty liver disease irrespectively of diet's total antioxidant capacity

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ABSTRACT

It is well established that oxidative stress is implicated in nonalcoholic fatty liver disease pathogenesis, whereas the dietary intake of antioxidants has been reported to be low in patients with the disease. We hypothesized that blood redox status measurements would be associated with nonalcoholic fatty liver disease presence and severity, and that diet's total antioxidant capacity could moderate the aforementioned association. The study sample consisted of 73 patients with nonalcoholic fatty liver disease, of which 58 were matched by age, sex, and body mass index with 58 controls. Diet's total antioxidant capacity was estimated through the ferric-reducing antioxidant power, the total radical-trapping antioxidant parameter, and the Trolox equivalent antioxidant capacity scores, whereas blood redox status was assessed by measuring thiobarbituric acid reactive substances levels, the enzymatic activity of glutathione peroxidase, and serum resistance to oxidation. Diet's total antioxidant capacity scores and glutathione peroxidase activity were not significantly associated with the disease presence or severity. Both thiobarbituric acid reactive substances and serum resistance to oxidation were significantly associated with the likelihood of nonalcoholic fatty liver disease (odds ratios [ORs], 7.769 [*P*= .007] and 0.936 [*P*= .033], respectively), independently of abdominal fat level, degree of insulin resistance, blood lipid levels, markers of subclinical inflammation, and diet's total antioxidant

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; CI, confidence interval; FFQ, food frequency questionnaire; FRAP, ferric-reducing antioxidant power; GGT, γ-glutamyl-transferase; GPx, glutathione peroxidase; HDL, high-density lipoprotein; Lag time, serum resistance to oxidation; MedDietScore, Mediterranean Diet Score; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; OR, odds ratio; OS, oxidative stress; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive substances; TEAC, Trolox equivalent antioxidant capacity; TGF-β1, transforming growth factor β1; TNF-α, tumor necrosis factor α; TRAP, total radical-trapping antioxidant parameters.

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capacity, but not with the disease histologic severity or stage. Our results support the association between blood redox status and the likelihood of nonalcoholic fatty liver disease regardless of diet's total antioxidant capacity.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease, affecting approximately 20% to 30% of the worldwide population [1]. It is currently considered as the hepatic manifestation of the metabolic syndrome, whereas its clinical spectrum is wide, ranging from simple fatty liver, defined as the hepatic triglyceride accumulation in the absence of excess alcohol intake, to nonalcoholic steatohepatitis (NASH), which can further lead to advanced liver fibrosis and cirrhosis [2]. Although still poorly understood, NAFLD pathogenesis is widely recognized as a "2-hit" model [3]. The "first hit" is a metabolic disturbance, mainly in relation to insulin resistance, that leads to hepatic steatosis and makes the liver vulnerable to the "second hit," including oxidative stress (OS) and dysregulations of proinflammatory cytokines, that triggers the progression of simple fatty liver to NASH.

Mounting evidence suggests that OS, defined as a persistent imbalance between the formation of pro-oxidant molecules and antioxidant defences, in favor of the former, is implicated in NAFLD pathogenesis [4]. The accumulation of free fatty acids in the liver leads to an elevated rate of fatty acid oxidation and an increased production of reactive oxygen and nitrogen species by mitochondria, microsomes, and peroxisomes. Reactive oxygen and nitrogen species can cause damage to hepatocytes, both directly via macromolecule peroxidation and indirectly via promoting inflammatory responses [5,6]. This increased state of intrahepatic OS can gradually deplete liver antioxidant resources and eventually lead to liver fibrogenesis and cell death via several mechanisms, including ATP depletion, hepatic stellate cells activation, and Kupffer cells stimulation [4].

The involvement of OS in NAFLD pathogenesis is further supported by studies assessing NAFLD patients' blood redox status. Most of the available data support an increase in blood oxidative markers in NAFLD patients and their positive association with disease severity, as well as an imbalance in both enzymatic and nonenzymatic blood antioxidant markers [7]. An insufficient dietary antioxidant intake could also contribute to this state of increased OS. Indeed, according to case-control studies, NAFLD patients report lower dietary intake of specific nutrients with antioxidant properties, namely vitamin C, vitamin A, vitamin E, and selenium, compared with healthy subjects [8,9]. However, no effort has been made so far to evaluate diet's total antioxidant capacity (TAC) in a sample of patients with NAFLD, as well as its possible moderating effect on the association between OS markers and the presence or severity of the disease.

We hypothesized that a state of increased OS would be positively associated with NAFLD presence and histologic stage, and that diet's TAC could moderate the aforementioned association. Therefore, we aimed to explore the relationship between blood redox status measurements and the presence or severity of NAFLD, after adjusting for dietary antioxidant intake to test its potential moderating effect. For the evaluation of blood redox status, both the degree of lipid peroxidation and the activity of antioxidant enzymes were measured. For the assessment of dietary antioxidant intake, diet's TAC was chosen over individual antioxidant nutrients because it better reflects the capacity of known and unknown antioxidants present in foods and beverages, as well as their synergistic interaction.

2. Methods and materials

A detailed description of the study population has been previously reported [10,11]. Briefly, the study sample consisted of 73 patients with a recent NAFLD diagnosis, based on elevated alanine aminotransferase (ALT) and/or γ-glutamyl transpeptidase (GGT) levels, evidence of hepatic steatosis on ultrasound, and exclusion of other causes of liver steatosis or injury, who visited the outpatient liver clinics of the 2nd Academic Department of Internal Medicine at Hippokration General Hospital of Athens. Reliable liver stiffness measurements by transient elastography (FibroScan, Echosens, France) were also available for 58 patients, whereas liver biopsy was performed in 34 patients who were further classified as having simple liver steatosis or NASH, based on the overall pattern of liver injury and the criteria of Brunt et al [12] modified by Kleiner et al [13]. Patients who had changed their dietary habits since diagnosis, those following a weight loss diet, and those with diabetes mellitus or any diagnosed malignancy were excluded from the study. A control group was also stratified, including 58 subjects with normal glucose metabolism and liver enzymes, no evidence of steatosis on ultrasound, and stable dietary habits during the last year that were matched with 58 of the above-mentioned patients according to age, sex, and body mass index (BMI). The study was approved by the Ethics Committee of the Hippokration General Hospital of Athens and by the Ethics Committee of Harokopio University and was conducted in accordance with the Declaration of Helsinki. All participants were informed about the aims and procedures of the study and gave their written consent.

A thorough medical history, anthropometric indices (BMI, waist circumference), abdominal fat compartments, physical activity habits, arterial blood pressure, as well as smoking habits and reception of any medication were recorded for all participants at enrollment, as previously described [10,11]. Habitual dietary intake over the last 12 months was assessed through a semiquantitative food frequency questionnaire (FFQ) enriched with foods and beverages commonly consumed in Greece [14], and participants' adherence to the Mediterranean diet through the Mediterranean Diet Score