European Journal of Nutrition
https://doi.org/10.1007/s00394-018-1776-0

ORIGINAL CONTRIBUTION

Association between dietary total antioxidant capacity and hepatocellular ballooning in nonalcoholic steatohepatitis: a cross-sectional study

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Received: 7 November 2017 / Accepted: 11 July 2018
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Abstract

Purpose Nonalcoholic steatohepatitis (NASH) is characterized by steatosis, lobular inflammation, ballooning, and in some cases, fibrosis, which can progress to cirrhosis and carcinoma. The progression of NASH is closely related to oxidative stress. Dietary intake of antioxidants has been suggested in protection against oxidative damage and related clinical complications. Thus, we evaluated the potential association of dietary total antioxidant capacity (TAC) with disease severity in NASH patients, as well as with anthropometric and body composition markers and biochemical parameters.

Methods Thirty-three outpatients with a mean age of 48.4 ± 1.9 years were evaluated. Dietary TAC was estimated from a quantitative food frequency questionnaire. NASH severity, determined by liver biopsy, lifestyle characteristics, occurrence of comorbidities, anthropometry, body composition, and biochemical parameters were assessed.

Results NASH patients who had a higher dietary TAC had fewer ballooned hepatocytes compared to those with a lower TAC ($p = 0.024$). The patients with the highest dietary TAC had a reduction of approximately 20% in the risk of having many ballooned hepatocytes (OR 0.791; 95% CI 0.643–0.974; $p = 0.027$). There was no association of steatosis, lobular inflammation, and fibrosis with dietary TAC. The same occurred for lifestyle characteristics, occurrence of comorbidities, anthropometry, body composition, and biochemical parameters.

Conclusion Dietary TAC is higher in patients with lower hepatic injury (ballooning), suggesting a possible role of food intake naturally high in its antioxidant capacity in reducing free radical production and, consequently, oxidative stress.

Keyword Total antioxidant capacity · Dietary antioxidants · Oxidative stress · Nonalcoholic steatohepatitis · Hepatocellular ballooning

Introduction

Nonalcoholic steatohepatitis (NASH) is a progressive, complex, and multifactorial liver disease [1]. It is characterized by the presence of steatosis, inflammation, hepatocyte injury (ballooning), and in some patients, progressive fibrosis, without significant alcohol consumption [2]. It is the most severe form of nonalcoholic fatty liver disease (NAFLD) that resembles alcohol-induced liver injury and can lead to cirrhosis and hepatocellular carcinoma. The main cause for NASH, previously described in the literature, is the consumption of hypercaloric diets, probably due to excess glucose, fructose and saturated fatty acids in combination with sedentary lifestyle [3–6].

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Published online: 17 July 2018

The actual prevalence of NASH is not fully known, since the disease is usually asymptomatic and the definitive diagnosis is only possible by histological evaluation [7, 8]. About 2–3% of the general population is estimated to have NASH [1]. Among morbidly obese individuals, about 70% are affected by this condition [9]. NASH is a hepatic expression of metabolic syndrome, due to its association with obesity, insulin resistance, and dyslipidemia [10].

The pathogenesis of NASH is not yet fully elucidated, but there are some hypotheses for the development of the disease [11–14]. In a "multiple hits" scenario, a very recent hypothesis, NASH pathogenesis is initiated through the triggering of excessive oxidative stress mediated by lipotoxic metabolites and additional pathogenic factors from other organs, such as gut-derived endotoxins resulting from increased gut permeability and gut dysbiosis, adipokines secreted from adipose tissue. This, in turn, drives hepatocyte death, inflammation, and fibrosis [2, 14]. Genetic variation, such as polymorphisms of patatin-like phospholipase 3 (PNPLA3), is also an important factor that can determine whether an individual has a high risk of developing NASH [15].

In fact, oxidative stress is considered a factor that contributes to the progression of NASH, since it elevates lipid peroxidation in cell membranes, stellate cell activation in the liver, leading to liver fibrosis, chronic inflammation, and apoptosis. Reactive oxygen species (ROS) and lipid peroxidation cause direct damage to hepatocytes by affecting membranes, proteins, and DNA. Thus, when antioxidant and anti-inflammatory defenses are becoming exhausted, a chronic state of steatohepatitis arises [16].

The total antioxidant capacity (TAC) of foods, which describes the ability of dietary antioxidants to scavenge preformed free radicals, has been suggested as a tool to investigate the health effects of antioxidants present in mixed diets. However, it is unclear whether diets with a high dietary TAC can modify oxidative stress, low-grade inflammation, or liver dysfunction, all of which are risk factors for obesity, diabetes, cardiovascular and liver disease [17–19].

A complex interaction between diet, lifestyle, and genetics is involved in NASH [20, 21]. Although there is no doubt about the importance of nutrition, evaluation of the dietary TAC of NASH patients has not been reported in the literature so far. We, therefore, conducted a cross-sectional study to examine the potential association of dietary TAC with disease severity in NASH patients, as well as with anthropometric and body composition markers and biochemical parameters.

Experimental methods

Study design

This cross-sectional study was conducted in the Gastroenterology/Hepatology Service of the University Hospital at the Federal University of Juiz de Fora, Minas Gerais, Brazil. Approximately 40 outpatients with NAFLD are attended monthly. The study was conducted from July 2015 to April 2017. The inclusion criteria were NASH patients over 18 years of age, of both sexes, low alcohol consumption, and available for anthropometric measurements and liver biopsy. Exclusion criteria included other causes of liver disease diagnosed as chronic hepatitis B and C, autoimmune hepatitis, hemochromatosis, Wilson's disease, and hepatocellular carcinoma. In addition, patients infected with the human immunodeficiency virus (HIV 1 or 2) and those using hepatotoxic drugs were not included in the study. The subjects were taken into the study after they provided written informed consent. This study was conducted in accordance with the Declaration of Helsinki. All procedures involving human subjects/patients were approved by the Ethics Committee of the Federal University of Juiz de Fora under protocol number 1.129.516 and all participants provided written informed consent.

Data collection

Information on age, sex, consumption of multivitamin supplements, alcohol, smoking, and physical activity were obtained from face-to-face interviews. Alcohol consumption was assessed with questions about the types of alcoholic beverages, the frequency of consumption, and the usual quantity consumed on each occasion. A daily intake of less than 20 g of alcohol (women) and 30 g (men) was adopted as a cut-off point for inclusion in the study, according to the American Gastroenterological Association [22]. Physical activity was determined through the Portuguese version of the International Physical Activity Questionnaire (IPAQ) validated by Pardini et al. [23]. Patients were classified as sedentary or active, according to the IPAQ. The diagnoses of diabetes, systemic arterial hypertension, and dyslipidemia were obtained from medical records. The presence of metabolic syndrome was defined according to the International Diabetes Federation criteria [24].

Definition of NASH

The diagnosis of NASH was made based on clinical and laboratory profiles and liver biopsy, which are routine evaluations in the Gastroenterology/Hepatology Service of the

University Hospital at the Federal University of Juiz de Fora. All patients underwent liver biopsy according to an evaluation protocol developed by the Brazilian Group for the Study of NAFLD [25]. The biopsy was assessed using the histologic scoring system of the NAFLD activity score [26] by a single hepatic pathologist who was blind for both clinical and biochemical data. Hepatic biopsies were evaluated for steatosis (0–3), lobular inflammation (0–3), hepatocellular ballooning (0–2), and staging of fibrosis (0–4) [26].

Biochemical measurements

Blood collection was performed after fasting for 12 h. The blood was separated by centrifugation and analyzed immediately at the Laboratory of Clinical Analysis of the University Hospital, following the Gastroenterology/Hepatology Service protocols of conduct for evaluating NASH. Serum levels of total cholesterol, high-density lipoprotein (HDL), triglycerides, fasting glycemia, glycated hemoglobin, ferritin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase, total proteins, albumin, total and direct bilirubin were determined using standard laboratory methods in an autoanalyzer (Wiener Lab, model CT600i). Fasting insulin was determined by analyzer (Architect, model i1000SR). Low-density lipoprotein concentration was calculated as described by Friedewald et al. [27]. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to Matthews et al. [28].

Anthropometric measures and body composition

Weight, height, waist circumference, and body composition were assessed in the fasting state. Waist circumference was measured at the midpoint between the iliac crest and the last rib. Body fat was measured by bioimpedance analyzer (Biodynamics model 450, Seattle, WA, USA). All these measurements were assessed in the fasting state.

Estimation of dietary total antioxidant capacity

The usual diet was obtained through a quantitative food frequency questionnaire previously validated by Ribeiro et al. [29]. Daily food consumption was estimated as frequency versus portion size for each item consumed. All food questionnaires were analyzed by the same nutritionist. For the determination of dietary TAC, a previously published database [30] was used, combined with the supporting literature [31–38], using the ferric reducing ability of plasma (FRAP) method with a calibration curve made with ferrous sulfate, for the TAC determination of food. Intake evaluation was performed using a standard spreadsheet developed in Microsoft Excel® by adding individual TAC values from the FRAP assay of each food, and expressed as TAC in mmol/day. To assign a TAC value to foods not available in the articles and in the database, botanically similar food data were used. When TAC values for cooked foods were not available, TAC levels of fresh foods were considered for estimation purposes.

Statistical analysis

Normal distribution of the data was determined by the Shapiro–Wilk test. Comparisons of continuous variables were conducted using the parametric Student's *t* test or non-parametric Mann–Whitney *U* test. Categorical variables were compared using the Chi-square test or Fisher's exact test. To determine the influence of dietary TAC, patients were divided into two groups according to the median dietary TAC value: lower than and equal to 6.59 mmol/day or higher than 6.59 mmol/day. Odds ratio (OR) and the corresponding 95% CI were estimated by binary logistic regression test. A scatter plot was drawn to demonstrate the relationship of hepatocellular ballooning and dietary TAC. The contribution of each food group to the dietary TAC was calculated as the ratio of the antioxidant intake from that food group to the total intake from all foods. Data are presented as mean and SEM, or median and minimum and maximum. Categorical variables are expressed as relative (%) and absolute (*n*) frequencies. All statistical analyses were performed using SPSS, version 20.0 (SPSS Inc., Chicago, IL, USA). A 5% level of significance was used.

Results

During the study period, 121 patients were enrolled in the study. After exclusion of 88 patients without confirmation of NASH by liver biopsy, or with incomplete data, 33 patients participated in the study, and 51.5% ($n=17$) were male. Overall anthropometrics, body composition, and comorbidities are shown in Table 1.

Table 1 Baseline characteristics of study participants

	Full sample ($n=33$)
Age (years)	48.4 ± 1.9
BMI (kg/m^2)	31.0 ± 0.9
WC (cm)	96.2 ± 2.0
Body fat (%)	33.4 ± 1.4
Fat free mass (%)	65.0 ± 2.0
Diabetes	24.2% ($n=8$)
Hypertension	48.4% ($n=16$)
Dyslipidemia	84.9% ($n=28$)
Metabolic syndrome	54.6% ($n=18$)

Values are means ± SEM or column percentages (%)

According to lifestyle characteristics, such as physical activity practice, alcohol consumption, smoking and the use of multivitamin supplements, and the occurrence of comorbidities, no significant differences were found between these variables and dietary TAC (Table 2). The anthropometric, body composition, and biochemical values also did not differ between groups with lower or higher dietary TAC, as observed in Table 3.

Histological findings revealed that 45.5% ($n=15$) of the patients presented 34–66% of hepatocytes with steatosis, 39.4% ($n=13$) presented greater than 66 and 15.2% ($n=5$) between 5–33%. Lobular inflammation was observed in 69.7% ($n=23$) in score 1 (< 2 foci) and 30.3% ($n=10$) in score 2 (2–4 foci). Ballooning in many cells was observed in 51.5% ($n=17$) of the patients. Regarding fibrosis staging, 45.5% ($n=15$) had no fibrosis, 48.5% ($n=16$) presented perisinusoidal/portal/periportal fibrosis, 3% ($n=1$) bridging fibrosis, and 3% ($n=1$) cirrhosis. Statistical analysis required a grouping of categories, in which it was observed that patients who had a higher dietary TAC had fewer ballooned hepatocytes compared to those with a lower TAC. Steatosis, lobular inflammation, and fibrosis did not differ between the groups with lower or higher TAC (Table 4).

As there was an association of higher dietary TAC with a smaller number of ballooned hepatocytes, a binary logistic

Table 2 Lifestyle characteristics and comorbidities according to dietary TAC value of nonalcoholic steatohepatitis patients

	TAC ≤ 6.59 mmol/days ($n=17$)	TAC > 6.59 mmol/days ($n=16$)	p value*
Physical activity			
Sedentary	64.7% (11)	37.5% (6)	0.056
Active**	35.3% (6)	62.5% (10)	
Alcohol consumption			
No	64.7% (11)	68.8% (11)	0.805
Yes	35.3% (6)	31.2% (5)	
Smoking			
No	88.2% (15)	100% (16)	0.485
Yes	11.8% (2)	0	
Use of supplements[#]			
No	76.5% (13)	93.8% (15)	0.335
Yes	23.5% (4)	6.2% (1)	
Central obesity			
No	17.6% (3)	18.8% (3)	0.999
Yes	82.4% (14)	81.2% (13)	
BMI			
Eutrophy	11.8% (2)	18.8% (3)	0.656
Overweight	88.2% (15)	81.2% (13)	
Diabetes			
No	88.2% (15)	62.5% (10)	0.118
Yes	11.8% (2)	37.5% (6)	
Arterial hypertension			
No	52.9% (9)	50% (8)	0.866
Yes	47.1% (8)	50% (8)	
Dyslipidemia			
No	17.6% (3)	12.5% (2)	0.999
Yes	82.4% (14)	87.5% (14)	
Metabolic syndrome			
No	41.2% (7)	50% (8)	0.732
Yes	58.8% (10)	50% (8)	

Nominal variables are given as the number of patients with the characteristic of interest

TAC total antioxidant capacity, *BMI* body mass index

*Fisher's exact test or Chi-square test. Differences were considered when $p<0.05$

**Irregularly active or active, according to IPAQ [23]

[#]Supplements: Calcium and Vitamin D

Table 3 Clinical markers according to dietary total antioxidant capacity (TAC) value of nonalcoholic steatohepatitis patients

	TAC ≤ 6.59 mmol/days ($n=17$)	TAC > 6.59 mmol/days ($n=16$)	p value*
BMI (kg/m^2)	31.0 ± 1.4	30.9 ± 5.2	0.990
WC (cm)	95.7 ± 3.3	96.6 ± 9.8	0.836
Body fat (%)	33.7 ± 1.8	33.2 ± 9.0	0.847
Fat free mass (%)	63.0 ± 3.1	66.5 ± 9.2	0.395
AST (UI/L)	51.0 (25–288)	42.5 (33–164)	0.871
ALT (UI/L)	54.0 (23–181)	57.0 (28–130)	0.900
GGT (UI/L)	52.0 (21–336)	66.0 (22–362)	0.665
AP (UI/L)	67.5 ± 5.9	82.4 ± 28.3	0.118
TB (mg/dL)	0.7 (0.3–2.5)	0.5 (0.3–1.3)	0.242
DB (mg/dL)	0.2 (0.1–1.0)	0.2 (0.1–0.6)	0.212
Total proteins (g/dL)	7.5 ± 0.1	7.5 ± 0.4	0.846
Albumin (g/dL)	4.3 (3.2–4.7)	4.4 (3.8–5.2)	0.239
Glucose (mg/dL)	98.0 (64–226)	98.5 (83–157)	0.367
Glycated hemoglobin (%)	5.8 (5.1–9.2)	5.6 (4.6–16.6)	0.999
Insulin (μU/ml)	12.5 (3.6–42.5)	11.8 (6.6–28.4)	0.482
HOMA-IR	3.0 (0.8–8.0)	2.9 (1.50–8.9)	0.719
TG (mg/dL)	176.0 ± 21.6	163.6 ± 55.2	0.632
TC (mg/dL)	189.1 ± 10.7	196.9 ± 37.7	0.590
HDL-c (mg/dL)	45.7 ± 3.2	43.6 ± 7.4	0.575
LDL-c (mg/dL)	111.4 (51–164)	117.9 (73–175)	0.313
Ferritin (ng/mL)	327.1 (109–593)	220.3 (42.4–615)	0.801

Continuous variables are given as median and minimum and maximum (in parentheses) or mean ± SEM

BMI body mass index, *WC* waist circumference, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma-glutamyltransferase, *AP* alkaline phosphatase, *TB* total bilirubin, *DB* direct bilirubin, *HOMA-IR* homeostasis model assessment-insulin resistance, *TG* triglycerides, *TC* total cholesterol, *HDL* high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol

*Student's t test or Mann–Whitney U test. Differences were considered when $p<0.05$

Table 4 Nonalcoholic steatohepatitis severity according to dietary total antioxidant capacity (TAC) value

	TAC ≤ 6.59 mmol/d ($n = 17$)	TAC > 6.59 mmol/d ($n = 16$)	p value*
Steatosis			
5–66%	47.1% (8)	75% (12)	0.157
> 66%	52.9% (9)	25% (4)	
Lobular inflammation			
< 2 foci	70.6% (12)	68.8% (11)	0.909
2–4 foci	29.4% (5)	31.2% (5)	
Hepatocellular ballooning			
Few cells	29.4% (5)	68.8% (11)	0.024
Many cells	70.6% (12)	31.2% (5)	
Fibrosis			
Absence	47.1% (8)	43.8% (7)	0.849
Presence	52.9% (9)	56.2% (9)	

Nominal variables are given as the number of patients with the characteristic of interest

*Fisher's exact test or Chi-square test. Differences were considered when $p < 0.05$

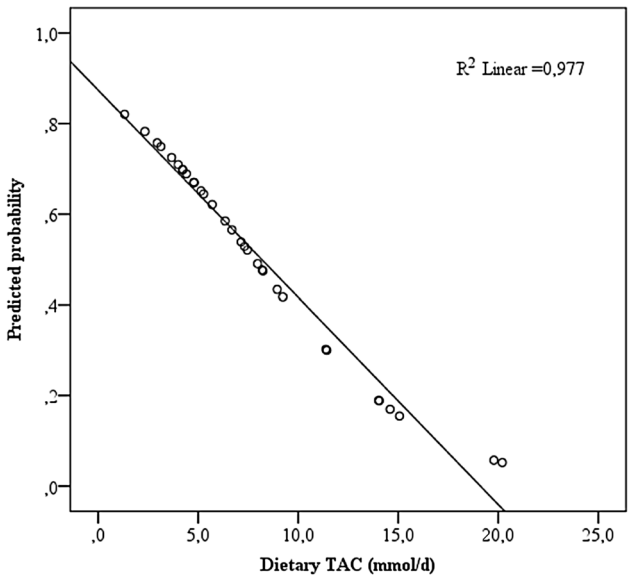

Fig. 1 Predicted probability of hepatocellular ballooning with increasing levels of dietary total antioxidant capacity (TAC) in nonalcoholic steatohepatitis patients (with 95% CI)

regression analysis was performed in which it was observed that with the increase of TAC there is a reduction of approximately 20% in the risk of having many ballooned hepatocytes (OR 0.791; 95% CI 0.643–0.974; $p = 0.027$). We can see a linear decrease in the predicted probability of having a ballooned hepatocyte, the higher the TAC, that is, between the two variables there is a negative association (Fig. 1).

Coffee and tea, fruits and fruit juices, vegetables, and legumes were the major food or food groups of dietary TAC (42.8, 29.2, 9.0, and 4.6%, respectively), while vegetable oils and nuts only accounted for less than 2%.

Discussion

It has been suggested that ingestion of dietary antioxidants may protect against oxidative damage and related clinical complications [39–41]. To our knowledge, this is the first study to evaluate dietary TAC and the relationships with NASH characteristics. The main finding of our study was that a higher TAC was associated with a smaller number of ballooned hepatocytes in NASH patients.

The pathogenesis of NASH and its progression to fibrosis is complex. Lipotoxicity, defined by excessive free fatty acid accumulation within hepatocytes, can lead to generation of toxic metabolites and cause hepatocyte injury via ballooning and, consequently, the initiation of NASH. Ballooned hepatocytes represent a cardinal histologic feature of lipotoxic hepatic injury and the magnitude of ballooned hepatocytes correlates with disease severity [2]. The accumulation of fat droplets in ballooned hepatocytes combines several pathogenic mechanisms in NASH including oxidative fat injury, endoplasmic reticulum stress, and abnormalities of the cytoskeleton in hepatocellular ballooning [42]. Oxidative stress is the result of a disturbance between the prooxidant/antioxidant balance and it can mediate liver injury through at least two major mechanisms: direct cell injury and indirect changes of cell signaling pathways. ROS induces activation of nuclear factor κB (NF-κB), a master regulator in the production of proinflammatory cytokines including interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), and interleukin-6 (IL-6) [2, 43, 44].

In fact, patients with NASH present an impaired metabolism of glutathione (antioxidant defense) when compared to healthy individuals [45]. Erhardt et al. [46] observed lower plasma levels of α-tocopherol, lutein, zeaxanthin, lycopene, α-carotene, and β-carotene in NASH patients. According to the authors, the reasons for this observation may be a depletion of antioxidants to counterbalance oxidative stress, or a reduced oral intake of food sources of nutrients or compounds with antioxidant activity. In this context, antioxidants may play an important role in protecting the hepatocyte cell membrane, inhibiting the proliferation of lipid peroxidation induced by ROS, and steatohepatitis progression [47].

The influence of dietary factors and potential interaction with oxidative stress has been highlighted in studies with NAFLD/NASH patients. Musso et al. [48] observed that dietary intake of nonobese, nondiabetic normolipidemic NASH patients was richer in saturated fat and cholesterol and was poorer in polyunsaturated fat, fiber, and antioxidant vitamins C and E, which led to the conclusion that dietary habits can promote steatohepatitis directly by modulating hepatic

triglyceride accumulation and reducing antioxidant activity. On the other hand, Georgoulis et al. [49] found no significant association between dietary TAC and NAFLD/NASH presence or severity. The authors evaluated anthropometric, lifestyle, laboratory, and clinical parameters. It is important to emphasize that the role of dietary factors in relation to oxidative stress phenomena in NASH patients has not yet been fully evaluated. The cross-sectional nature of many studies does not allow a causal relationship to be established between oxidative stress and NASH development, as well as the emergence of oxidative stress as a primary hit in the pathogenesis of the disease.

There is a growing search in the literature about the potential for antioxidant therapy in NAFLD/NASH, considering the potential for beneficial effects in treatment. Despite this promise, antioxidants have produced mixed results in several clinical trials [50, 51]. The most promising results involve vitamin E therapy [47]. Vitamin E was superior to placebo for improvement in hepatocellular ballooning and NAFLD activity score in children, but no effects were observed on hepatic or portal fibrosis, nor on lobular inflammation [52]. Vitamin E was superior to placebo for the treatment of NASH in adults without diabetes [53]. The authors observed a significant improvement in serum alanine and aspartate aminotransferase levels, hepatic steatosis, and lobular inflammation, but no improvement in fibrosis scores. In another study, antioxidant therapy using both vitamins C and E was conducted with NASH patients [54]. Serum alanine aminotransferase, thioredoxin, and high-sensitivity C-reactive protein levels, and liver histology were clearly improved with vitamin C and E therapy. It should be emphasized that methodological problems, such as sample size, duration of follow-up, insufficient parameters, and heterogeneous nature of the supplemented antioxidant compounds lead to a complicated interpretation of the results of the clinical trials.

In the present study, dietary TAC was similar between sexes, and was not associated with any of the lifestyle, anthropometric, body composition, and biochemical variables. The patients evaluated are attended on an out-patient basis and have regular medical follow-up with drug therapy, which may be reflected in their clinical status.

The pathogenesis of NASH and its potential progression to fibrosis, cirrhosis, and hepatocellular carcinoma occur in response to a chronic inflammatory state with insulin resistance, hepatic steatosis, and oxidative stress [2, 55]. The ability to treat a disease relies heavily on the knowledge of disease etiology. So far, the main treatment options for NAFLD/NASH that involve lifestyle modification include following a healthy diet, associated with weight loss, and practicing physical activity [2, 56]. The present study reports an inverse association between TAC values in the diet and hepatocellular ballooning in NASH patients, suggesting a possible role of dietary antioxidant intake in hepatocyte homeostasis. Thus, to reduce the production of free radicals and to attenuate oxidative stress, increasing the consumption of foods with higher antioxidant capacity, such as coffee, tea, fruits, vegetables, and legumes, is suggested [41, 57–59]. In the present study, coffee and tea were the main contributors to dietary TAC, which is not surprising, considering previous results by Torres and Farah [18] with the Brazilian diet. Given that foods contain many different types of antioxidants (vitamins, carotenoids, polyphenols, and other still unknown bioactive compounds), their TAC has been suggested as a better tool for investigating the relationship between a diet's antioxidant potential and oxidative stress-induced diseases [49].

A strong point of our study was the selection of NASH patients well-characterized through the clinical, laboratory, and histological profile. Liver biopsy evaluation is the gold standard for the detection of NASH and is considered very useful in differentiating NASH from other diseases [60, 61]. The limitations of the present study include the cross-sectional study design which does not permit the assessment of temporal relationships.

Taken together, our results suggest that the preference for foods with naturally elevated antioxidant capacity may be a simple and potential approach in the nutritional treatment of NASH. Further studies are needed addressing food consumption with long-term antioxidant potential to confirm our findings and assess the benefit of food interventions in NASH, considering that the action of nutrients is synergistic and interactive.

Acknowledgements The study was supported by Brazilian Government Organization (Fundação de Amparo à Pesquisa do Estado de Minas Gerais/FAPEMIG; APQ 01522-15). D. G. O. is the recipient of a CAPES grant. D. G. O., F. F. G., and (A) P. (B) M. participated in the design and protocol of the study. D. G. O., F. F. G., and J. M. O. carried out the study. D. G. O. and H. H. M. F. participated in the evaluation of the dietary total antioxidant capacity. D. G. O., (A) P. (B) M., and L. E. V. V. (C) F. were involved with editing the manuscript and all authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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