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Dietary patterns in Brazilian patients with non-alcoholic fatty liver disease: a cross-sectional study

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OBJECTIVE: Recent evidence suggests that non-alcoholic fatty liver disease is associated with diet. Our aim was to investigate the dietary patterns of a Brazilian population with this condition and compare them with the recommended diet.

METHODS: A cross-sectional study was conducted on 96 non-alcoholic fatty liver disease patients before any dietetic counseling. All patients underwent abdominal ultrasound, biochemical tests, dietary evaluations, and anthropometric evaluations. Their food intake was assessed by a semi-quantitative food-frequency questionnaire and 24-hour food recall.

RESULTS: The median patient age was 53 years, and 77% of the individuals were women. Most (67.7%) participants were obese, and a large waist circumference was observed in 80.2% subjects. Almost 70% of the participants had metabolic syndrome, and 62.3% presented evidence of either insulin resistance or overt diabetes. Most patients (51.5, 58.5, and 61.7%, respectively) exceeded the recommendations for energy intake, as well as total and saturated fat. All patients consumed less than the amount of recommended monounsaturated fatty acids, and 52.1 and 76.6% of them consumed less polyunsaturated fatty acids and fiber, respectively, than recommended. In most patients, the calcium, sodium, potassium, pyridoxine, and vitamin C intake did not meet the recommendations, and in 10.5-15.5% of individuals, the tolerable upper limit intake for sodium was exceeded. The patients presented a significantly high intake of meats, fats, sugars, legumes (beans), and vegetables and a low consumption of cereals, fruits, and dairy products compared with the recommendations.

CONCLUSIONS: Although patients with non-alcoholic fatty liver disease exhibited high energy and lipid consumption, most of them had inadequate intake of some micronutrients. The possible role of nutrient-deficient intake in the development of non-alcoholic fatty liver disease warrants investigation.

KEYWORDS: Non-Alcoholic Fatty Liver Disease; Diet; Food; Obesity; Metabolic Syndrome; Brazil.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is currently recognized as one of the most common causes of chronic liver disease. It is usually associated with metabolic syndrome (MS) (1), which is characterized by numerous interrelated risk factors for cardiovascular disease. Obesity and diabetes are predictors of advanced liver fibrosis and cirrhosis in NAFLD patients (2). In the United States, the prevalence of obesity in 2007-2008 was 32.2% among adult men and 35.5% among adult women (3). In Brazil, 48% of adults were overweight in 2008-2009, and approximately 12.5% of men and 16.9% of women were obese (4). Because the prevalence of MS and obesity has increased in most countries, the burden of NAFLD is also expected to rise (5).

Lifestyle interventions, including dietary changes and increases in physical activity, are the first-line treatment for NAFLD (6). It has been suggested that dietary composition plays a role in NAFLD pathogenesis; thus, changing dietary patterns may constitute a therapeutic resource even in the

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absence of weight reduction (7). A few recent studies have been performed to address these questions, but their results are quite ambiguous (8-14). Some authors note a positive association of the increased consumption of cholesterol (8), saturated fatty acids (8), total fatty acids (12), and a high n-6/n-3 fatty acid ratio (12) with the presence of the disease. On the other hand, others report an association between NAFLD and a lower intake of polyunsaturated fats (8) and total lipids (13). Although a positive association of carbohydrate intake with NAFLD (10,13) and MS (14) was previously demonstrated, there is no consensus regarding the relationship between the type of carbohydrate ingested and NAFLD (9,14). Clearly, this issue warrants further investigation.

Considering the lack of knowledge regarding the diet of NAFLD patients in Brazil, we undertook this study to evaluate the dietary patterns of Brazilians with NAFLD and compare it with the Dietary Reference Intakes (15) and the Dietary Guide for Brazilians (16).

METHODS

A cross-sectional study was performed that included 96 of the 114 consecutive patients diagnosed with NAFLD in the Hepatology Clinic, University Hospital, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, during a two-year period (2007-2009). This institution is a reference center of the Brazilian public health system. The inclusion criteria comprised a diagnosis of NAFLD according to the American Gastroenterological Association criteria (17) and a lack of previous nutritional counseling.

The American Gastroenterological Association criteria for NAFLD include steatosis on an abdominal ultrasound and/or liver biopsy (performed according to clinical judgment), the exclusion of other causes of liver disease (namely alcohol intake >20 g/day, markers of chronic B or C hepatitis virus infections, auto-immune hepatic disorders, Wilson disease, hemochromatosis, and alpha-1-antitripsin deficiency), no use of steatogenic medications within the past six months, no exposure to hepatotoxins, and no history of bariatric surgery (17). Alcohol use was addressed on at least three different occasions, namely by two doctors and by a dietician during a nutritional interview. Physical activity was evaluated according to the patient's information. Considering the frequency, duration and type of physical activity (walking for recreation, moderate activity, or vigorous activity), our patients were classified as very active, active, irregularly active, or sedentary (18).

MS was defined according to the National Cholesterol Education Program's Adult Treatment Panel III (19).

The study was approved by the local ethics committee (ETIC 280/07), and all patients signed an informed consent form at the time of screening for the study.

Clinical, laboratory, ultrasound and histological investigations

All participants underwent anthropometric, laboratory and abdominal ultrasound investigations. The anthropometric data included height (m), weight (kg), body mass index (kg/m^2), and waist circumference (cm). Being overweight was defined as having a body mass index >25 and <30 kg/m^2, and obesity was defined as having a body mass index ≥30 kg/m^2 (20). A waist circumference ≥88 cm (women) or ≥102 cm (men) was defined as high (19).

The laboratory assessment included liver biochemistry, lipid profile, uric acid, TSH and fasting serum insulin and glucose analyses. Insulin sensitivity was calculated using the homeostasis model assessment (21), and insulin resistance was defined by homeostasis model assessment values ≥3 (22).

Fatty liver was diagnosed by abdominal ultrasound using standardized criteria (17). The abdominal ultrasound was performed in all subjects using the same equipment and by the same operator, who was unaware of the clinical and laboratory results. Some patients underwent a liver biopsy according to clinical judgment, and the diagnosis of non-alcoholic steatohepatitis was based on the accepted clinical-pathological criteria (23).

Dietary assessment

Dietary intake was assessed by 24-hour food recall (24h-FR) and a semi-quantitative food frequency questionnaire (FFQ) adapted to the Brazilian population, which served as a representation of the usual intake. The first method is a suitable tool with which to evaluate food and beverage intake within the previous 24 hours. It is easy to apply, is inexpensive, and does not depend on the respondent's literacy. The semi-quantitative FFQ includes a list of more than 80 food items and provides information on long-term nutritional habits. It is the most commonly used method to assess dietary intake in epidemiological studies, and its reproducibility and validity have been investigated in the Brazilian adult population in different studies (24,25). For each food item, the participants indicated their average frequency of consumption over the past year in terms of number of specified meal sizes consumed per day/weekly/biweekly/rarely/never. The selected frequency category for each food item was converted into a daily intake (9). The nutrient components of each food item were taken from the Brazilian Food Composition Table (26).

The dietary reference values for nutrient intake by Americans and Canadians described in the Dietary Reference Intakes guide (15) were used to evaluate the nutrient intake adequacy, taking into account the estimated average intake and the adequate intake. The estimated average intake corresponds to the amount of daily ingestion of a nutrient estimated to satisfy the needs of half of the individuals in a group. The adequate intake values, which are calculated to cover or exceed the needs of all members of a group, were used for nutrients without an available estimated average intake. Both the estimated average intake and the adequate intake take into consideration the individual's gender and age. The nutrient consumption over the tolerable upper intake limit was determined. The tolerable upper limit intake represents the maximum daily amount of a nutrient that appears to be safe for the majority of healthy people (15).

The Dietary Guide for Brazilians, which consists of guidelines proposed by the Brazilian Ministry of Health according to the recommendations of the World Health Organization, was used to evaluate food intake adequacy (16).

Groups of foods were classified according to the Dietary Guide for Brazilians as follows: 1) cereals, roots and tubers; 2) fruits and vegetables; 3) legumes (beans and other plant foods rich in protein); 4) milk and dairy products; 5) meat and eggs; and 6) fats and sugars (16).

Statistical analysis

Statistical analyses were performed using the STATA software, version 9.0 (StataCorp LP, College Station, TX, USA). The data are presented as frequencies, proportions, means and standard deviations, and medians. The Shapiro-Wilk test was used to determine whether continuous variables were normally distributed. Continuous variables were compared using the t-test (normal distribution) or the Mann-Whitney U test (asymmetrical distribution), and proportions were compared using the chi-square test or Fisher's exact test, where appropriate. To compare the nutrient intake with both the Dietary Reference Intakes and the Dietary Guide for Brazilians, the paired t-test or the Wilcoxon test was used for normal or asymmetrical distributions, respectively. For all tests, p-values <0.05 were considered statistically significant.

RESULTS

Characteristics of the study population

The demographic, anthropometric, and key laboratory data of the patients are presented in Table 1. Of the 96 patients, 74 (77%) were women, and the median age was 53 years (25th and 75th percentiles = 48 and 60 years, respectively). None of the participants was undernourished, four (4.2%) had normal weight, 27 (28.1%) were overweight, and 65 (67.7%) were obese according to the body mass index classification. A high waist circumference was observed in 77 (80.2%) individuals, namely, 15 men (68.2%) and 62 women (83.8%) ($p = 0.11$). Seventy-seven (80.2%) patients were sedentary, and 19 (19.8%) were irregularly active. Six (6.2%) individuals were smokers. Almost 70% of the study population had MS, and 60 individuals (62.3%) presented evidence of either insulin resistance (homeostasis model assessment values ≥ 3) or overt diabetes. HDL cholesterol was low in 11 (52.4%) men and 53 (75.7%) women ($p = 0.05$). Other clinical features are shown in Table 2.

Approximately 20% of the subjects underwent liver biopsy, and 13 (13.5%) of them had non-alcoholic steatohepatitis based on histological examination.

Adequacy of energy, macronutrient, and fiber intake

Table 3 shows the proportion of patients who met (or did not meet) the recommendations for energy, macronutrient, and fiber intake. According to both the 24h-FR and the semi-quantitative FFQ, all patients had a monounsaturated fat intake lower than the recommended amount, and more than 50% of them had a lower consumption of fiber and polyunsaturated fats than recommended. On the other hand, the intake of saturated fat and energy was higher than recommended in most patients. According to the semi-quantitative FFQ evaluation, the carbohydrate intake was below and the total fat intake was above the recommended values.

Adequacy of vitamin and mineral intake

The proportions of patients who met (or did not meet) the recommended amounts of vitamins and minerals are presented in Table 4. Both the 24h-FR and the semi-quantitative FFQ evaluations demonstrated that almost all patients had a calcium intake lower than the recommended value. Most individuals had a low consumption of potassium, vitamin C, and pyridoxine. According to the 24h-FR evaluation, 10.6% of the subjects exceeded the tolerable upper limit intake of sodium, and according to the FFQ evaluations, the amount of sodium consumption was higher than recommended in 15.5% of the cases. Based on the 24h-FR results, more than 50% of the patients had a low intake of retinol, thiamin, magnesium, and manganese.

Food intake adequacy

The dietary data concerning food group intake are summarized in Table 5. There were differences in the intake of all food groups compared with the guide. The average number of consumed meals that included cereals, fruits, and dairy products was lower than the recommended number. However, the number of meals consumed with legumes (beans), vegetables, meats, fats, and sugar was higher than recommended in the Dietary Guide for Brazilians.

DISCUSSION

Our NAFLD patients recalled a diet richer in lipids (specifically saturated fat and energy) and poorer in monounsaturated fat, polyunsaturated fat, fiber, calcium, potassium, vitamin C, and pyridoxine than the recommendations. A large proportion of this population also exceeded

Table 1 - Demographic, anthropometric, and laboratory data of NAFLD patients.

	Total	*Male*	*Female*	
Parameters	*Median/Mean (range)*	*Median/Mean (range)*	*Median/Mean (range)*	*p-value*
Age (years)	54.4 (29.0; 81.0)	56.6 (36.0; 70.0)	53.7 (29.0; 72.0)	0.23[a]
Weight (kg)	80.4 (53.5; 130.8)	87.2 (63.0; 96.4)	78.4 (53.5; 107.5)	0.008[a]
BMI (kg/m^2)	32.2 (23.4; 44.7)	31.0 (34.3; 31.1)	32.6 (24.4; 41.6)	0.16[a]
Glucose (mg/dl)	98.0 (64.0; 387.0)	94.0 (77.0; 275.0)	98.0 (64.0; 387.0)	0.48
Insulin (μU/ml)	10.1 (2.0; 23.5)	9.2 (2.0; 15.8)	10.4 (3.9; 24.0)	0.50[a]
HOMA	1.9 (0.5; 6.3)	1.7 (0.5; 3.7)	2.0 (0.8; 6.3)	0.60
Total cholesterol (mg/dl)	211.0 (120.0; 423.0)	187.0 (139.0; 291.0)	216.0 (120.0; 423.0)	0.03
Triglycerides (mg/dl)	173.0 (61.0; 620.0)	156.0 (61.0; 618.0)	178.0 (75.0; 620.0)	0.60
AST (x RV)	0.7 (0.3; 4.9)	0.7 (0.5; 1.8)	0.7 (0.3; 4.9)	1.00
ALT (x RV)	0.6 (0.1; 3.3)	0.6 (0.3; 2.4)	0.6 (0.1; 3.3)	0.50
AP (x RV)	0.8 (0.2; 2.7)	0.7 (0.33; 1.1)	0.8 (0.2; 2.7)	0.30
GGT (x RV)	1.1 (0.3; 15.5)	1.0 (0.4; 7.8)	1.1 (0.3; 15.5)	0.51
Albumin (g/dl)	4.4 (3.7; 5.2)	4.5 (3.9; 5.0)	4.4 (3.7; 5.2)	0.61[a]

NAFLD, non-alcoholic fatty liver disease; BMI, body mass index; WC, waist circumference; HOMA, homeostasis model assessment; HDL, high-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AP, alkaline phosphatase; GGT, gamma-glutamyltransferase; RV, reference value. Means were compared using the t-test ([a]), and medians were compared using the Mann-Whitney test.

Table 2 - Clinical characteristics of NAFLD patients.

Parameters	*Total (%)*	*Male (%)*	*Female (%)*	*p-value*
Glucose intolerance or diabetes	43.8	36.4	46.0	0.43
HOMA ≥3 (without diabetes)	22.5	20.0	23.0	1.00[a]
Insulin resistance	62.3	55.6	64.1	0.51
Hypercholesterolemia	60.4	45.5	64.9	0.10
Low HDL	69.6	52.4	75.7	0.05
Hypertriglyceridemia	63.2	57.1	64.9	0.52
Hypertension	70.8	63.6	73.0	0.40
Hyperuricemia	11.7	19.1	9.6	0.26[a]
Hypothyroidism	11.8	9.5	12.5	1.00[a]
Metabolic syndrome	69.5	52.3	74.3	0.05

NAFLD, non-alcoholic fatty liver disease. Proportions were compared using the chi-square test or Fisher's exact test ([a]).

the tolerable upper limit for sodium intake. These findings correspond to a higher consumption of meats, fats, and sugar groups, with a lower intake of cereals, fruits, and dairy products.

Interestingly, and in contrast to the results obtained by Cortez-Pinto et al. (12), all of our patients reported low monounsaturated fat consumption. Most of them did not consume any foods rich in monounsaturated fats, and less than 12% reported olive oil intake. The relationship between a high intake of monounsaturated fat and a good lipid profile (reduction of low-density lipoprotein (LDL) cholesterol and triglycerides) is well known, and the beneficial effects of a diet rich in monounsaturated fats, particularly a diet in which monounsaturated fats replace both saturated fat and high amounts of carbohydrates, have been extensively investigated (27). As previously reported (8), we also observed a significantly high consumption of total and saturated fat. The intake, corresponding to approximately five and two times the recommendations, respectively, of foods belonging to the ''rich in fat group'' and the ''meat group'', certainly contributed to these findings.

Although our patients recalled recent low and adequate long-term carbohydrate consumption, their daily intake of meals containing the ''sugar group'' was approximately twice the recommended intake. It is well known that a high carbohydrate intake plays an important role in the pathogenesis of NAFLD. Furthermore, the available evidence suggests that the type of carbohydrate ingested may also influence NAFLD pathogenesis (9,14,27). Foods with a high glycemic index (e.g., sugar) increase *de novo* hepatic lipogenesis, hypertriglyceridemia, hepatic insulin resistance, and liver steatosis (27).

Despite their high vegetable consumption, most of our NAFLD population reported low dietary fiber and vitamin C intake. These findings may be due to the low intake of fruits.

Approximately 40% of our patients had a high protein intake, which is in agreement with the high ''meat group'' consumption. Similar results were described by Zelber-Sagi et al. (9). Although a direct association between NAFLD and protein intake has never been investigated, there are reports of an association between protein intake and both insulin resistance and diabetes (27).

The higher energy consumption in relation to the estimated requirements, determined by both recent and long-term assessments, most likely reflects the increased intake of food from the ''sugar group'' and the ''fat group''. According to some authors, patients with non-alcoholic steatohepatitis have a higher energy intake compared with a control group (11-14). Even in healthy people, hypercaloric food intake could be related to alanine aminotransferase abnormalities (28).

Calcium intake was below the recommended value in more than 94% of our population, most likely due to the reduced consumption of milk and dairy products. Recent studies have suggested that calcium deficiency is related to obesity by increasing lipogenesis and hyperinsulinemia and inhibiting lipolysis (29). Furthermore, prospective studies have also suggested that the intake of dairy products and calcium reduces the risk of obesity, abdominal obesity, diabetes, hypertension, and MS (29,30). The magnesium intake was also below the recommended value in most of our patients. As magnesium deficiency plays a role in increasing insulin resistance in diabetes and MS patients (31), we can speculate that this mineral may have some effect on the pathogenesis of NAFLD. Although the magnesium intake of 13.8% of our patients was above the recommended value, the effects of excessive magnesium consumption are known only for magnesium derived from a pharmacological agent, not when the source is food and water (15).

Table 3 - Distribution of NAFLD patients by category of adequacy / inadequacy of energy, macronutrient and fiber intake.

Parameters	Recommendation		24-hour food recall				Food frequency questionnaire			
			Intake Amount	Adequate	Lower	Over	Intake Amount	Adequate	Lower	Over
	Male	Female	Median/ Mean (range)	(%)	(%)	(%)	Median/ Mean (range)	%	%	%
Energy	EER	EER	2084.8 (1116.2; 4212.1)	0.0	48.9	51.1	2507.9 (473.9; 6575.3)	0.0	32.1	67.9
Carbohydrate[a]	50-60% TEI		270.5 (101.9; 485.7)	28.7	53.2	18.1	320.3 (49.0; 1150.2)	38.1	34.5	27.4
Protein	0.8-1.0 g/kg		75.4 (5.7; 178.2)	22.3	34.0	43.7	73.5 (21.3; 238.8)	22.6	32.1	45.2
Total fat	25-35% TEI		82.9 (32.4; 178.5)	36.2	5.3	58.5	85.1 (17.7; 203.7)	44.1	14.3	41.7
Saturated fat	<10% TEI		22.8 (7.6; 82.2)	0.0	38.3	61.7	20.9 (4.5; 64.5)	0.0	33.3	66.7
MUFA[a]	<20% TEI		23.2 (7.3; 45.4)	0.0	100.0	0.0	24.1 (6.2; 73.2)	0.0	100.0	0.0
PUFA[a]	<10% TEI		24.1 (1.8; 50.5)	0.0	52.1	47.9	25.8 (4.2; 81.5)	0.0	54.8	45.3
Fiber	20-30 g		18.2 (6.3; 56.5)	2.1	76.6	21.3	24.3 (8.0; 76.3)	0.0	53.6	46.4

NAFLD, non-alcoholic fatty liver disease; EER, energy expenditure requirements; TEI, total energy intake; MUFA, monounsaturated fat; PUFA, polyunsaturated fat. Mean ([a]).

Table 4 - Distribution of NAFLD individuals by category of adequacy / inadequacy of mineral and vitamin intake.

Parameters	Recommendations		24-hour food recall				Food frequency questionnaire			
			Intake Amount	Adequate	Lower	Over	Intake Amount	Adequate	Lower	Over
	Male	Female	Mean/Median (range)	(%)	(%)	(%)	Mean/Median (range)	(%)	(%)	(%)
Magnesium (mg)[a]	≤30 yr: 330 >30 yr: 350	≤30 yr: 255 >30 yr: 265	183.8 (74.3; 534.3)	0.0	86.2	13.8	349.7 (95.4; 2696.3)	0.0	34.5	65.5
Manganese (mg)	2.3	1.8	1.6 (0.2; 13.8)	0.0	68.1	31.9	2.4 (0.6; 9.6)	0.0	32.1	67.9
Phosphorus (mg)	34		858.1 (178.5;1995.3)	0.0	0.0	100.0	1003.6 (294.6;2791.2)	0.0	0.0	100.0
Iron (mg)	6.0	19-50 yr: 8.1 >50 yr: 5.0	7.1 (2.4; 19.4)	0.0	33.3	66.7	7.4 (2.9; 21.1)	0.0	35.7	64.3
Sodium (g)[a]	≤50 yr: 1.5 51-70 yr: 1.3 >70 yr: 1.2		1.2 (0.0; 3.8)	2.1	66.0	31.9	1.3 (0.2; 3.7)	0.0	56.0	44.1
Potassium (g)[a]	4.7		2.0 (0.9; 5.4)	0.0	97.9	2.1	2.6 (1.0; 7.0)	0.0	95.2	4.8
Copper (µg)	700		654.1 (209; 3004.4)	0.0	39.4	60.6	1257.2 (380.9; 5442.5)	0.0	40.0	60.0
Zinc (mg)	9.4	6.8	8.8 (1.4; 25.4)	0.0	29.8	70.2	8.0 (2.4; 30.6)	0.0	39.3	60.7
Vitamin A (µg)	625	500	125.0 (0.0; 1275.2)	0.0	91.6	8.4	267.4 (1.4; 5337.5)	0.0	38.5	61.5
Thiamine (mg)	1.0	0.9	0.7 (0.1; 2.6)	0.0	68.1	31.9	1.1 (0.3; 4.0)	0.0	38.1	61.9
Riboflavin (mg)	1.1	0.9	1.0 (0.0; 3.7)	0.0	46.8	53.2	1.2 (0.2; 4.1)	1.2	35.7	63.1
Pyridoxine (mg)	≤50 yr: 1.3 >50 yr: 1.7	≤50 yr: 1.3 >50 yr: 1.5	0.7 (0.1; 3.7)	0.0	76.6	23.2	0.9 (0.1; 4.7)	0.0	64.3	35.7
Niacin (mg)	12	11	14.7 (0.5; 37.7)	0.0	31.9	68.1	14.0 (3.4; 57.9)	0.0	34.5	65.4
Calcium (mg)[a]	≤50 yr: 1000 >50 yr: 1200		18.0 (0.0; 444.0)	0.0	76.6	23.4	79.2 (2.5; 620.6)	0.0	95.2	4.8
Vitamin C (mg)	75	60	360.0 (13.8; 1213.3)	0.0	100.0	0.0	472.0 (108.6; 1734.4)	0.0	94.1	5.9

NAFLD, non-alcoholic fatty liver disease. Recommendations are expressed as the adequate intake ([a]) or as the estimated average intake.

Sodium intake was most likely underestimated in our sample population, as we took into account only the amount of sodium naturally contained in food but not the addition of sodium. This limitation was due to the difficulties in measuring the addition of sodium to foods and preparations. Nevertheless, 10.5-15.5% of our patients exceeded the upper limit intake of sodium. It is well known that this dietary pattern increases the risks of hypertension, cerebrovascular disorders, and coronary heart disease (32,33).

Our NAFLD patients reported a low consumption of antioxidant vitamins, specifically vitamins A and C. The low intake of retinol is at least partially due to the reduced consumption of milk (average, 0.4 meals per day) and eggs (average, 0.04 meals per day). The low intake of fruits, as mentioned previously, may have contributed to the low intake of vitamin C and beta-carotene, a precursor of vitamin A. These findings are consistent with the results of an Italian study, which demonstrated the low consumption of vitamin C by non-alcoholic steatohepatitis patients (8). Additionally, low serum levels of retinol in NAFLD individuals were observed in a Brazilian investigation (34). It is well known that antioxidant vitamins have a protective role against oxidative stress (34); therefore, the supplementation of these vitamins for treating NAFLD has been widely investigated (35).

In this study, the high zinc, iron, copper, phosphorus, riboflavin, and niacin consumption above the estimated average intake and adequate intake values may be related to the high intake of the ''meat group'' (average, 0.7 meals/1,000 kcal). Moreover, the high consumption of beans, a typical food in Brazilian cuisine, may also explain the high iron and cooper intake. Contrary to our observation, Toshimitsu et al. (14) found low zinc consumption by NASH individuals. Additionally, in accordance with our findings, Alla and Bonkovsky (36) demonstrated a high intake of iron, especially heme-iron, which plays a role in NAFLD pathogenesis by increasing oxidative stress. In this context, some authors observed that the dietary restriction of iron, calories, and fat is associated with a decrease in serum aminotransferases and ferritin levels in NAFLD (37). The data from a recent study suggest that the dietary restriction of copper in rats may be involved in the development of hepatic steatosis and insulin resistance (38). In our study, in contrast, the intake of copper was above the recommended level. However, the association between NAFLD and copper intake has never been investigated in humans. To our knowledge, there are no published data relating phosphorus, niacin and riboflavin to NAFLD.

Our study has some methodological limitations. As this study was a cross-sectional investigation, it is not possible to associate dietary patterns with the relative risk of NAFLD. However, the study provides the first description of the dietary patterns of Brazilian patients with NAFLD. In this context, a control group would have added more information regarding possible differences (or lack of differences) in relation to the dietary patterns of Brazilians without NALFD. We did not stratify our population according to age and socioeconomic level, which are factors that could influence dietary patterns. However, most of our subjects (66%) were aged between 40 and 59 years, and almost all of them (98%) were aged between 30 and 70 years; furthermore, it is well known that patients utilizing the public health system in Brazil have low purchasing power. It

Table 5 - Comparison between the number of meals consumed by NAFLD patients containing various food groups and the recommended numbers.

Food groups	Recommendation per 1000 kcal	Median/Minimum (range)	*p*-value
Cereals, roots and tubers	0.3 meals/day	2.1 (0.4; 4.4)	<0.00005[a]
Legumes (beans and other plant foods rich in protein)	0.5 meals/day	0.6 (0.0; 3.9)	0.0 [a]
Vegetables	1.5 meals/day	1.6 (0.0; 12.4)	0.01
Fruits	1.5 meals/day	0.9 (0.0; 7.4)	<0.00005
Milk and dairy products	1.5 meals/day	0.4 (0.0; 1.9)	<0.00005
Meat and eggs	0.5 meals/day	0.6 (0.0; 2.1)	0.003
Fats	0.5 meals/day	2.4 (0.3; 5.5)	<0.00005[a]
Sugars	0.5 meals/day	1.0 (0.0; 4.5)	<0.00005

NAFLD, non-alcoholic fatty liver disease. Non-normally distributed variables were compared using the Wilcoxon test, and normally distributed variables were compared using the paired t-test ([a]).

would be better if the diagnosis of NAFLD had been established by liver biopsy in all patients. To minimize this limitation, we rigorously followed the American Gastroenterology Association criteria (17). Additionally, the possibility of both memory and reporting bias in dietary assessment should not be ruled out, especially when considering individuals, such as obese subjects, who have knowledge regarding ''healthy diets". The omission of information on food consumption has been reported by several authors (39-42). Furthermore, all methods for dietary pattern evaluation are subject to sampling error. Thus, to minimize these potential errors, we used two methods to assess food intake. The FFQ questionnaire is one of the most accepted methods for measuring food consumption in epidemiological studies, as it is simple, fast and reliable.

In conclusion, our NAFLD patients had excessive saturated fat, total lipid and energy intake, as well as some nutritional deficiencies, such as monounsaturated fat, polyunsaturated fat, calcium, vitamin C, and pyridoxine intake deficiencies. These dietetic features could be related to the poor long-term consumption of grains, fruits and dairy products, as well as to the consumption of too many meals containing legumes (beans), meats, fats and sugars, compared with the recommendations of the Dietary Guide for Brazilians. The possible role of each nutrient deficiency in the development of NAFLD needs further investigation.

AUTHOR CONTRIBUTIONS

Ferolla SM, Couto CA, Couto OF and Ferrari TC conceived and designed the study. Ferolla SM, Lima ML and Reis TO collected the data. Tavares-Jr WC performed the abdominal ultrasounds. Vidigal PV performed the pathological analysis and disease classification (non-alcoholic fatty liver disease or non-alcoholic steatohepatitis). Fausto MA performed the statistical analysis. Ferolla SM, Couto CA, and Ferrari TC prepared the manuscript. All authors have read and approved the final version of the manuscript.

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