RESEARCH ARTICLE

Non-alcoholic Fatty Liver Disease and Its Relationship with the Nutritional Status of Vitamin A in Individuals with Class III Obesity

Gabriela Villaça Chaves · Silvia Elaine Pereira · Carlos José Saboya · Andréa Ramalho

Abstract

Background The objective of the present study was to investigate vitamin A nutritional status in individuals with class III obesity through a biochemical indicator (retinol and β-carotene serum levels), correlating these findings with non-alcoholic fatty liver disease (NAFLD) presence and its risk factors.

Methods The studied population was composed of 145 patients with morbid obesity [body mass index, BMI≥ 40 kg/m^2) of both sexes. Retinol and β-carotene serum levels were assessed by high performance liquid chromatography. The cutoff values used for serum retinol and β-carotene inadequacy were <1.05 μmol/l and ≤40 μg/dl, respectively. Insulin resistance (IR) was assessed through homeostasis model assessment index (HOMA) method. Biochemical parameters of liver enzymes, lipid profile, and glycemia were analyzed. Anthropometric measurements were conducted. NAFLD diagnosis was performed through magnetic resonance.

Results NAFLD prevalence in the group was 71%. An inadequacy of 11.3 and 41.7% of retinol and β-carotene serum levels, respectively, was found when NAFLD was present. A significant correlation of serum retinol with albumin liver and total bilirubin was found. As regards β-carotene, a positive correlation for HDL-c variable and a negative correlation for the HOMA-IR, weight, and BMI variables were observed. There was a significant association between IR presence and retinol and β-carotene inadequacy.

Conclusion The high inadequacy of retinol and β-carotene nutritional status in the sample, with a higher inadequacy in those with NAFLD, suggests an increase in the utilization of vitamin A in this group related to the fight against the oxidative stress to what they are exposed to. The significant association between retinol and β-carotene with IR supports the hypothesis that vitamin A may have a protector effect on IR pathogenesis.

Keywords Vitamin A · Oxidative stress · Non-alcoholic fatty liver disease · Insulin resistance

G. Villaça Chaves · S. E. Pereira · C. J. Saboya · A. Ramalho
Micronutrients Research Group,
Instituto de Nutrição Josué de Castro,
Universidade Federal do Rio de Janeiro,
Rio de Janeiro, Brazil

G. Villaça Chaves (✉)
Av. Pau-Brasil, 211-Bl. J – Ilha da Cidade Universitária,
Rio de Janeiro, RJ 21949-900, Brazil
e-mail: gabrielavc@gmail.com

Introduction

Non-alcoholic fatty liver disease (NAFLD) presents a comprehensive histological aspect which results from the deposit of triglycerides into hepatocytes. It embodies a status of pathological alterations similar to those observed in alcoholic hepatopathies but occurring in non-alcoholic individuals. These alterations vary from simple steatosis to non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis [1].

Although diagnosed all over the world, NAFLD has variances in prevalence. In the USA, a country where one fourth of the adult population is obese, steatosis reaches more than two thirds of those individuals and more than 90% of class III obese [2]. The actual incidence is still unknown and probably higher than what is assumed, as it presents a silent clinical course, unspecific laboratory alterations, and in its initial stages, liver biopsy and/or

ultrasound are/is not performed in individuals who belong to this disease risk group.

Predominance of the female sex and association with obesity, type II diabetes mellitus, and hyperlipidemia are classical aspects of this disease [3]. Primary NAFLD is related to the presence of obesity, type II diabetes mellitus, and hyperlipidemia, whereas secondary NAFLD is caused by use of some drugs and toxins, surgeries for obesity, total parenteral nutrition, among others [4].

The rise of obesity may be verified as much in the richest social classes as in the poorest populations, as the industrialization process not only favored sedentary life-styles but also reduced the cost of foods, thus, making more accessible those foods poor in vitamins and minerals with high content of energy and fat, especially the saturated ones [5]. Because of this, we may observe coexistence between obesity and malnutrition, which suggests nutritional problems that superpose such as obesity and deficiency of micronutrients, therefore, characterizing the so-called hidden hunger.

Hidden hunger is the term utilized for characterizing micronutrient deficiency, and it is defined as the non-explicit lack of one or more micronutrients, besides being recognized as the most prevalent nutritional issue in the world. It is the previous stage of the manifestation of clinical signs of these lacks, and it is not necessarily associated with diseases clearly defined as those observed in energetic and proteic malnutrition. In this context, vitamin A deficiency (VAD) is inserted, being considered one of the health public issues most prevalent in the world, as it brings several damages to the health of individuals, including death [6].

Vitamin A participates in several functions that are primordial to the human system, acting in visual acuity, in cellular proliferation and differentiation, and immunological activity [7]. Retinol and carotenoids have received a prominent position for their role against reactive oxygen species (ROS), protecting the organism against oxidative stress (OS) and, consequently, preventing damages and tissue lesions related to several chronic diseases [8].

Considering the role of OS in NAFLD pathogenesis and the potent action of vitamin A in the fight against ROS, it is probable that individuals with this disease will have lower levels of this vitamin, as OS much increases the intake of substances with antioxidant function [9]. Moreover, risk factors for NAFLD, as obesity, dyslipidemias, and hyperglycemia, isolatedly, have demonstrated an association with decreased levels of vitamin A [10].

The aim of the present work was to investigate the nutritional status of vitamin A in individuals with BMI≥ 40 kg/m^2 through a biochemical indicator (serum levels of retinol and β-carotene), relating these findings to NAFLD presence and to insulin resistance (IR).

Materials and Methods

The studied population was composed of 145 patients with class III obesity (BMI≥40 kg/m^2), of both sexes, attended in a private clinic in the city of Rio de Janeiro. The study comprised all patients who met the inclusion criteria and who had been attended in the clinic in the period from January through December 2006. The clinic referred to attends about 240 patients per year with surgical indication for weight loss. Overall, the studied population was composed of approximately 60% of the total annual attendance.

Exclusion criteria were: patients with disabsorptive syndromes, severe and chronic infections, pregnant and lactating women, and patients with associated endocrinopathies who made use of medication/supplement containing vitamin A or who consumed alcoholic beverages superior to 20 g/day in women and 40 g/day in men. Individuals who had any other liver disease than NAFLD were also excluded.

For diagnosing NAFLD, magnetic resonance imaging test was applied.

Weight and height indicators were utilized for assessing nutritional status. BMI was measured according to the following formula: actual body weight (kg)/height (m^2) [11]. The adopted cutoffs were those recommended by WHO [12] for classification of eutrophy and overweight, and the participants were classified in class III obesity with BMI≥40.0 kg/m^2. The present study proposed the categorization of the individuals in BMI subclasses, and the sample was distributed in 5 kg/m^2 intervals, which originated five ranges: range 1 for those between 40 and 44.9 kg/m^2, range 2 between 45 and 49.9 kg/m^2, range 3 between 50 and 54.9 kg/m^2, range 4 between 55 and 59.9 kg/m^2, and range 5 for those between 60 and 64.9 kg/m^2.

For determining fat body distribution, waist and hip circumferences were measured. Waist-hip ratio (WHR) was determined according to the following equation: WHR= waist circumference/hip circumference.

A 5-ml blood sample was obtained by vein punction of patients in a 12-h fast for determining the serum levels of retinol and β-carotene. High performance liquid chromatography–ultra violet was the method used for retinol and β-carotene quantification. As the obtained serum values of retinol were compared with normality cutoffs proposed by WHO [13], they were presented in 0.35-μmol/l intervals. In the present study, the serum retinol value of ≥1.05 μmol/l was considered as adequate, and <1.05 μmol/l was the cutoff utilized to indicate VAD [14]. As suggested by Sauberlich et al. [15], the cutoff to indicate inadequacy of serum values of carotenoids was lower or equal to 40 μg/dl.

IR was determined by the homeostasis model assessment index (HOMA) method [16] through the calculation where HOMA-IR=insulinemia after fast (mU/l)×glycemia after fast (mmol/l)/22.5. The cutoff utilized for IR was 4.0,

identified by the receiver operating characteristic (ROC) curve as the one with best sensitivity and specificity for individuals in this study.

Other laboratory tests were performed for assessment of lipid profile and biochemical parameters of liver enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), total bilirubin (TB), albumin, prothrombin time test (PTT), cholesterol, triglycerides, HDL, LDL, besides glycemia and basal insulin.

At the time of the individuals' enrollment in the study, diagnoses of diabetes mellitus and systemic arterial hypertension were carried out by a physician in charge of this according to norms of the V Brazilian Guidelines of Arterial Hypertension [17]. Diagnosis of diabetes mellitus was carried out according to norms of the American Association of Diabetes [18].

Statistic analyses were performed in the SPSS for Windows version 10.0 statistic package. Measures of central tendency and dispersion were calculated besides the median and minimum and maximum values of the continuous variables. The distribution of the referred values was identified as not normal. For multiple comparisons of the numeric variables among three or more groups, the Kruskal–Wallis variance analysis was performed. Comparison of the numeric variables between two groups was performed by Mann–Whitney test. Associations between the categorical variables were performed either by the chi-quadrate test χ^2 or by Fisher exact test. Contingence coefficient was provided to measure the degree of association among the variables. ROC curve was utilized to determine the best cutoff for HOMA-IR variable.

The present study was approved by the Ethics Committee on Research of Hospital Universitário Clementino Fraga Filho of Universidade Federal do Rio de Janeiro/UFRJ.

Results

The sample was composed of 145 individuals, 44 (30.3%) men and 101 (69.7%) women.

Mean age was 36.5±11.7 years, varying from 19 to 64 years, with 37.5% of the sample in the range of 19–30 years, 47.8% in the range of 31–50 years, and 14.7% of the individuals in the range of 51–70 years according to IOM (2001) classification. There was no significant difference between mean age and BMI related to gender.

Mean age was significantly higher in the group with NAFLD diagnosis, being 38.2±11.5 and 32.2±11.1 years in the group with and without steatosis, respectively (p=0.006).

The prevalence of NAFLD in the group studied was 71%, with positive diagnosis in 75.0 and 69.3% of males and females, respectively, with no statistical difference (p=0.553).

The prevalence of arterial hypertension, dyslipidemia, and diabetes mellitus/intolerance to glucose in the group with steatosis was 56.9, 81.6, and 68.0%, respectively.

An association between the presence of IR and NAFLD diagnosis was found, and 75.5% of the individuals with NAFLD diagnosis presented inadequacy of HOMA-IR index. Besides, 85% of the individuals without the disease had adequate levels of HOMA-IR (p<0.001).

As regards the nutritional status of vitamin A, the individuals studied presented a mean of 1.66±0.65 μmol/l for serum levels of retinol and mean of 53.8±32.7 μg/dl for serum levels of β-carotene. Distribution of the sample according to serum levels of retinol showed that 12.7% of the patients were below the <1.05 μmol/l cutoff, which characterizes inadequate levels of vitamin A. Among the patients with inadequate serum levels of retinol, 10.4% were in the ≥0.70 μmol/l<1.05 μmol/l class, indicating marginal deficiency, whereas 2.3% were concentrated in the >0.35 μmol/l<0.70 μmol/l class, indicating moderate deficiency. Of the patients, 37.5% showed inadequate serum levels of β-carotene (≤40 μg/dl). In the comparison of means between the group with and without the disease, β-carotene presented a mean significantly lower in the group with NAFLD (47.2±26.2 and 70±40.9 in the group with and without the disease, respectively; p=0.003). Retinol did not present significant mean difference between the individuals with and without the disease (1.63±0.6 and 1.73±0.8, respectively; p=0.487).

No significant association was found between the adequacy of serum retinol in patients with and without NAFLD (p=0.311). An inadequacy of 11.3 and 41.7% of levels of retinol and β-carotene, respectively, was found when NAFLD was present. Only 26.8% of the individuals without NAFLD showed β-carotene inadequacy (p=0.068).

Comparing β-carotene means according to serum retinol adequacy in individuals with NAFLD, it was possible to observe retinol progressive decrease as the severity of VAD increased (Fig. 1). It is important to point out that β-carotene mean was significantly higher in the group with adequate retinol when compared with those groups with marginal VAD (adjusted p=0.01) and moderate (adjusted p=0.01). No significant difference was found between the serum levels of β-carotene between the groups with marginal and moderate VAD.

In the correlation of serum levels of retinol and β-carotene with biochemical and anthropometric variables according to NAFLD diagnosis, as what regards retinol, in patients with NAFLD, the biochemical variables as albumin (r=0.30; p=0.002), triglycerides (r=0.20; p=0.042), and very low-density lipoprotein (VLDL; r=0.47; p=0.001) showed a coefficient of positive correlation with statistical significance. The TB variable showed a negative correlation coefficient with statistic significance (r=−0.352;
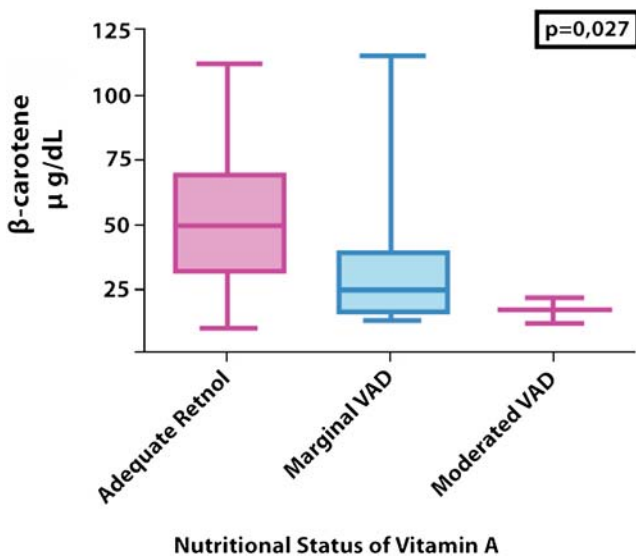
Fig. 1 Distribution of means of β-carotene according to the nutritional status of Vitamin A

$p=0.001$). No correlation was found between the biochemical variables in the patients without steatosis. No correlation was observed between retinol and the anthropometric variables independent of the NAFLD diagnosis. In individuals without the disease, no significant correlation was found with any of the variables studied.

As regards β-carotene, a positive correlation with statistic significance for the HDL variable was observed ($r=0.254$; $p=0.010$) in patients with NAFLD. The HOMA-IR index presented a significant negative correlation ($r=-0.301$; $p=0.002$). The anthropometric variables such as weight ($r=-0.239$; $p=0.015$) and BMI ($r=-0.198$; $p=0.045$) presented negative correlation with statistic significance in these patients. No correlation was observed between β-carotene and the studied variables in the individuals without the disease.

In the comparison of the means of the biochemical and anthropometric variables between the groups with adequate and inadequate retinol, the individuals who had adequate retinol presented a significantly higher mean of VLDL and albumin. Besides, those with adequate retinol presented lower means with statistical significance of TB and basal insulin. No significant difference was observed for the anthropometric variables (Table 1).

When comparing the means of the biochemical and anthropometric variables between the groups with adequate and inadequate β-carotene, the patients who showed β-carotene adequacy presented means significantly lower for the weight anthropometric variable and for the HOMA-IR index. Mean HDL was significantly higher in those individuals (Table 2).

No association was observed between the nutritional status of retinol and β-carotene and the presence of diabetes

Table 1 Comparison of means of the biochemical variables between the groups with adequate and inadequate serum retinol according to NAFLD diagnosis

Biochemical and anthropometric parameters	Adequate serum retinol≥1.05 μmol/l		Inadequate serum retinol <1.05 μmol/l		p value
	Mean±SD	Median (min–max)	Mean±SD	Median (min–max)	
Weight (kg)	126.9±23.1	65.64 (87.6–192.0)	128.0±18.6	80.32 (95.5–155.8)	0.524
BMI (kg/m2)	45.4±5.4	67.07 (40.0–63.4)	46.1±5.4	70.44 (40.0–55.1)	0.620
WC (cm)	126.2±15.1	64.30 (97.0–165.0)	128.7±16.4	77.38 (111.0–160.0)	0.740
HC (cm)	135.8±12.5	65.99 (101.0–166.0)	131.8±12.9	62.26 (110.0–153.0)	0.393
RWH	0.92±0.9	18.67 (0.7–1.1)	0.98±0.9	23.92 (0.8–1.1)	0.206
AST (U/l)	25.7±10.2	67.75 (12.0–68.0)	24.4±8.6	65.76 (14.0–44.0)	0.820
ALT (U/l)	34.6±20.0	67.65 (8.0–132.0)	38.6±19.0	66.50 (17.0–78.0)	0.463
AST/ALT	0.85±0.32	67.93 (0.4–2.3)	0.69±0.16	64.53 (0.3–0.9)	0.118
GGT	39.5±28.6	67.89 (10.0–200.0)	39.3±27.2	64.82 (16.0–109.0)	0.991
Total bilirubin (mg/dL)	0.40±0.22	41.68 (0.1–0.6)	0.58±0.15	71.25 (0.2–1.2)	0.015
Albumin (g/dL)	4.4±3.8	70.28 (2.5–4.0)	3.7±0.37	44.65 (3.0–4.3)	0.005
PTTs	0.21±0.62	23.78 (0.6–1.7)	0.3±0.42	17.25 (0.2–1.2)	0.532
Cholesterol (mg/dL)	201.6±43.5	66.61 (120.0–239.0)	206.3±32.2	73.62 (143.0–260.0)	0.463
Triglycerides (mg/dL)	153.8±81.4	69.04 (42.0–574.0)	130.4±36.8	56.91 (85.0–208.0)	0.509
HDL (mg/dL)	46.5±11.9	66.84 (22.0–71.0)	44.4±5.8	64.18 (37.0–54.0)	0.765
LDL (mg/dL)	120.5±39.8	63.04 (53.0–240.0)	135.2±33.0	81.82 (85.0–200.0)	0.097
VLDL	30.9±14.1	48.91 (8.0–71.0)	20.8±4.2	29.68 (17.0–28.0)	0.024
Glucose (mg/dL)	100.7±27.1	66.11 (72.0–213.0)	101.6±14.8	77.06 (86.0–130.0)	0.285
Basal insulin (μU/mL)	22.0±12.8	65.96 (2.5–72.2)	29.5±16.3	70.18 (11.2–71.4)	0.05
HOMA-IR	4.3±2.6	58.96 (1.1–7.8)	5.0±1.7	69.25 (1.3–17.3)	0.076

Mann–Whitney test.

SD Standard deviation, *PTTs* seconds above control.

Table 2 Comparison of mean biochemical variables between the groups with adequate and inadequate β-carotene according to NAFLD diagnosis

Biochemical and anthropometric parameters	Adequate serum β-carotene ≥40 mg/dl		Inadequate serum β-carotene <40 mg/dl		p value
	Mean±SD	Median (min–max)	Mean±SD	Median (min–max)	
Weight (kg)	123.7±21.8	67.9 (95.5–181.0)	131.7±21.8	80.0 (87.6–192.0)	0.011
BMI (kg/m^2)	44.7±4.9	68.0 (40.0–59.1)	46.5±5.7	80.0 (40.0–63.4)	0.073
WC (cm)	124.8±13.7	67.6 (106.0–164.0)	128.8±16.5	75.3 (97.0–165.0)	0.165
HC (cm)	133.8±11.2	68.3 (103.0–165.0)	136.9±13.5	72.7 (97.0–165.0)	0.246
WHR	0.93±0.9	17.0 (0.7–0.1)	0.93±0.9	22.8 (0.7–1.1)	0.416
AST (U/l)	25.6±9.5	73.4 (12.0–57.0)	26.4±11.3	69.5 (14.0–68.0)	0.857
ALT (U/l)	35.5±19.5	71.0 (10.0–112.0)	38.0±23.2	73.6 (8.0–132.0)	0.683
AST/ALT	0.81±0.28	74.65 (0.4–2.0)	0.81±0.36	67.50 (0.3–2.3)	0.514
GGT	39.4±30.3	71.9 (10.0–200.0)	43.0±29.2	72.0 (15.0–171.0)	0.549
Total bilirubin (mg/dl)	0.55±0.22	69.9 (0.2–1.1)	0.57±0.22	76.8 (0.1–1.2)	0.639
Albumin (g/dl)	4.0±0.41	78.7 (2.5–4.9)	4.8±5.5	60.9 (3.0–4.0)	0.087
PTTs	0.21±0.42	27.1 (0.2–1.2)	0.32±0.94	28.3 (0.9–1.7)	0.801
Cholesterol (mg/dl)	202.0±42.3	71.9 (140.0–329.0)	200.6±41.6	73.5 (120.0–303.0)	0.886
Triglycerides (mg/dl)	155.5±88.1	69.4 (42.0–574.0)	145.2±55.3	77.6 (51.0–312.0)	0.968
HDL (mg/dl)	48.1±11.5	77.8 (27.0–71.0)	42.7±10.1	58.9 (22.0–68.0)	0.033
LDL (mg/dl)	120.7±38.0	69.3 (64.0–240.0)	124.0±39.5	69.8 (53.0–235.0)	0.660
VLDL	29.6±14.3	47.2 (8.0–71.0)	29.9±11.7	49.8 (17.0–52.0)	0.832
Basal glycemia (mg/dl)	104.8±30.4	76.8 (72.0–213.0)	94.0±14.0	65.1 (75.0–136.0)	0.091
Basal insulin (μU/ml)	22.8±13.8	72.3 (2.5–72.2)	22.3±12.3	70.1 (12.3–6.9)	0.865
HOMA-IR	4.3±0.9	56.3 (1.1–8.7)	4.6±1.5	73.41 (1.0–17.0)	0.030

Mann–Whitney test.

SD Standard deviation, *PTT* seconds above control.

mellitus, arterial hypertension, and dyslipidemia as much in the total sample as when we only considered the individuals with NAFLD. No significant difference was also found between adequacy of the levels of retinol and the BMI subclasses (5-kg/m^2 intervals) proposed by this study. A significant association was observed between the increase of BMI subclasses and β-carotene inadequacy in individuals with NAFLD ($p=0.05$).

As regards insulin resistance, considering the individuals with NAFLD, there was a significant association between the presence of IR and retinol and β-carotene inadequacy, with 100% of the individuals with low levels of serum retinol presenting inadequacy of the HOMA-IR index ($p=0.029$). Concerning β-carotene, an inadequacy of 97.6% was observed in individuals resistant to insulin ($p<0.001$). No significant association was found between the presence of IR and retinol and β-carotene adequacy in individuals without diagnosis of NAFLD.

Discussion

In the present study, mean age of the individuals with NAFLD diagnosis despite being low was significantly higher when compared with individuals without the disease. Studies have demonstrated high prevalence of NAFLD in younger age ranges [19, 20], which comes accordingly to the rise of obesity in more and more young classes of the population [5].

The prevalence of steatosis found in this study was 71%, similar to that found by Crespo et al. [19] who observed 70% of steatosis in a sample of morbidly obese. In class III obesity, steatosis has been shown in almost the overall individuals (85–90%), with the presence of NASH in about 25–70% [21].

Although the sample was predominantly composed of women, the prevalence of steatosis was higher in men (75%). The first studies related to the epidemiology of NAFLD point to a higher prevalence of the disease in women [22]. Nonetheless, recent studies have demonstrated a higher prevalence of NAFLD in men, further showing that this disease affects all ethnic and racial groups [2, 23, 24]. As it is a disease that has been most studied lately, there is still the need of a better understanding of some issues related to its epidemiology. A higher prevalence of steatosis in men might be explained by a higher tendency of abdominal fat accumulation in men than in women [23].

Obese individuals seem to be in higher risk for presenting decreased levels of serum retinol or carotenoids. Studies have shown lower serum levels of carotenoids in obese when compared with eutrophic individuals [25]. The prevalence of inadequate serum levels of retinol found in the present study was 12.7%, with β-carotene inadequacy in 37.5% of the sample.

This is the first study which is addressed to investigate the prevalence of VAD in individuals with class III obesity with NAFLD. Although no significant association was found between adequacy of serum retinol in patients with and without NAFLD, it was possible to observe a higher inadequacy of β-carotene in those patients with the disease (41.7%) when compared with those who did not have the diagnosis of the disease (26.8%) and with the overall group (37.5%). Besides, in the comparison of the means between the groups of individuals with and without NAFLD, a significantly lower mean of β-carotene was found (p= 0.003) in the group with the disease (47.2±26.2 µg/dl and 70.0±40.9 µg/dl, respectively). It is probable that there is a bidirectional relationship between the nutritional status of this micronutrient and NAFLD: Hepatic steatosis may aggravate the nutritional status of β-carotene probably caused by a higher demand of antioxidant substances in such a degree that individuals with low serum levels of this micronutrient may have a higher risk for developing this disease.

In the present study, in individuals with NAFLD, it was possible to observe a progressive decrease of β-carotene as VAD severity increased. β-carotene is recognized as the most potent retinol precursor. The present study corroborates the findings of Mecocci et al. [26] showing that there was a higher mobilization of β-carotene for conversion into retinol according to VAD aggravation. This finding is worthy to be pointed out, as β-carotene is possibly being deviated from other important functions as the fight against OS to maintain an adequate nutritional status of retinol. Thus, it is important to guarantee the adequacy of the serum levels of retinol so as to preserve the function of β-carotene as antioxidant, especially in obese individuals with steatosis, taking into account the increased demand of antioxidant substances.

Albumin, TB, and PPT are tests that classically assess liver function [27]. Up to the present time, there are no studies assessing the relationship between these biochemical parameters of liver enzymes and NAFLD presence or severity. Generally, studies conducted with individuals with this disease only assess the markers of liver lesion such as AST, ALT, and GGT. Hence, this is the first study which assesses all tests of liver lesion and function, besides their relationship with serum levels of retinol and β-carotene in individuals with NAFLD.

In the present study, in individuals with NAFLD, the biochemical variables such as albumin, triglycerides, and VLDL showed positive correlation with retinol, whereas the TB variable showed negative correlation with statistical significance.

Rocchi et al. [28] and Newsome et al. [29] also evidenced a direct and significant correlation between serum retinol and serum albumin in individuals with chronic liver disease. Romagnuolo et al. [30] observed that albumin inferior to 35 g/l was an independent predictor of moderate and severe inflammation and of advanced fibrosis/cirrhosis. In the present study, serum levels of albumin were significantly higher in the group with adequate serum retinol. However, mean values, as much in the group with adequate retinol as in the group with inadequate retinol, were inside normality.

TB median values were significantly higher in the group with VAD, but the mean was inside normality in both groups. Rocchi et al. [28] found a negative and significant correlation between serum retinol and TB in individuals with cirrhosis, with 1.5 mg/dl mean TB, which is about three times higher than that found in the present study. Peres [31], studying individuals who were carriers of chronic liver disease of viral C etiology, also observed positive correlation between retinol and albumin and negative correlation between retinol and TB. Despite similar correlations found in the present study, as well significant difference between the means of these variables according to retinol adequacy, albumin and TB means were inside normality.

Previous studies which assessed the relationship between retinol and the tests of liver function were conducted in individuals with advanced liver disease (cirrhosis, hepatocellular carcinoma). In spite of the low severity of the disease studied in the present work, which may explain the reason why no relationship was found between retinol and other biochemical parameters of liver lesion and function besides the finding that mean values of the biochemical parameters were inside normality in the group with the disease, it is already possible to point the role of retinol as a potential marker of liver function. Nonetheless, it is still necessary to conduct studies that assess retinol as marker of liver function and liver lesion in simple steatosis and in more severe NAFLD forms.

As regards β-carotene, a positive correlation with statistical significance for the HDL variable with means of HDL significantly higher was observed in individuals with adequate β-carotene. This antioxidant acts as an important protector against the LDL oxidative attack and has also been associated with HDL increase in vitro and vivo [32]. It is important to point out that in the present study HDL, the only component of lipid fraction which is associated with the presence of NAFLD, was also the only one that showed correlation with β-carotene. Such findings suggest the involvement of β-carotene and HDL in the installation and aggravation of NAFLD as well in the development of other comorbidities associated with this disease. These findings still suggest a possible protective effect of β-carotene and of HDL on NAFLD pathogenesis.

The HOMA-IR index showed a significant negative correlation with higher means in individuals with adequate β-carotene. Besides, 97.6% of the individuals with low

levels of serum β-carotene had IR. As regards retinol, an inadequacy of 100% was observed in individuals resistant to insulin. Individuals with adequate retinol also presented lower means of basal insulin. Sugiura et al. [33] observed a reverse association between the serum levels of carotenoids and the estimated IR through the HOMA-IR method, which supports the hypothesis that carotenoids may have a protective effect in the pathogenesis of IR probably on account of its role as protective agent in OS, as it has been suggested that the increase of OS implicates in a decrease of insulin action [34]. Facchini et al. [35] reported a reverse correlation between α-carotene, β-carotene, and lutein with IR. Ford et al. [36] also observed a negative correlation between the serum levels of carotenoids and the levels of basal insulin in a study of population basis.

As regards anthropometric variables, weight and BMI evidenced a significant negative correlation with β-carotene corroborating the findings of Viroonudomphol et al. [37] who showed a negative correlation between retinol, β-carotene, and BMI. Moreover, studies performed in adult population reported lower levels of β-carotene in individuals with higher values of BMI similar to those of the present study where we found a significant association between the increase of BMI subclasses and β-carotene inadequacy in individuals with NAFLD.

Publications relating vitamin A to NAFLD are still scarce. The present study was the first to show the association of β-carotene with NAFLD and its risk factors, especially IR and HDL inadequacy; however, there is yet the need of establishing the relationship of the nutritional status of vitamin A in NAFLD different stages, associating this vitamin with this disease severity as it has already been shown in studies conducted with individuals with chronic liver disease of other etiologies, thus, opening a field for new researches.

Therefore, it is recommended that the assessment of retinol and serum β-carotene might be included in the attendance routine of NAFLD patients as a tool of clinical practice aiming to guarantee an adequate nutritional status of this vitamin in these individuals. It is also important to conduct studies which will utilize the histological assessment for this disease diagnosis so that it will be possible to establish retinol as a possible marker of liver function and lesion according to the disease severity, and, as well, to establish β-carotene, associated or not with HDL, as possible protective factors against this disease development.

References

1. Harrison AS, Bisceglie AM. Advances in the understanding and treatment on nonalcoholic fatty liver disease. Drugs 2003;63: 2379–94.

2. Ruhl CE, Everhart JE. Epidemiology of non-alcoholic fatty liver. Clin Liver Dis. 2004;8:501–19.

3. Ludwig J, Vigginao TR, Mc Gill DB. Nonalcoholic steatohepatitis. Mayo Clin Proc. 1980;55:434–8.

4. Matteoni CA, Younossi AM, Gramlich, T. Non-alcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413–9.

5. Instituto Brasileiro de Geografia e Estatística (IBGE). Pesquisa de Orçamento Familiar—POF 2002–2003. http://ftp.ibge.gov.br/Orcamentos_Familiares/Pesquisa_de_orcamentos_Familiares2002_2003/Precisão_das_Estimativas. Acesso em 09/08/2006.

6. WHO (World Health Organization). Global prevalence of vitamin A deficiency. WHO/NUT/95.3. Geneva: WHO; 1995

7. Institute of Medicine (IOM). Vitamin A. In: Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington: National Academy Press; 2001. p. 82–161.

8. Delport R, Ubbink JB, Human JA, et al. Antioxidant vitamins and coronary artery disease risk in South African males. Clin Chim Acta. 1998;278:55–60.

9. Sarni RO, Ramalho RA. Serum retinol and total carotene concentrations in obese children. Med Sci Monit. 2005;11:510–4.

10. Souza LB, Veiga GV, Ramalho RA. Níveis séricos de retinol e carotenóides e sua associação com o estado nutricional antropométrico em escolares e adolescentes. Rev SOCERJ 2004;17:147.

11. Cuppari L. Guias de Medicina Ambulatorial e Hospitalar. Nutrição Clínica no Adulto. UNIFESP. São Paulo: Manole; 2002.

12. World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO Consultation on Obesity. WHO: Geneva; 1998.

13. World Health Organization. Indicators for assessing vitamin A deficiency and their application in monitoring and evaluating intervention programmes. Micronutrient Series, WHO/NUT. 10. Geneva, Switzerland: World Health Organization; 1996.

14. IVACG (International Vitamin A Consultative Group). Improving the vitamin A status of populations. USAID-ILSI; 2003.

15. Sauberlich HE, Hodges RE, Wallace DL. Vitamin A metabolism and requirements in the human studied with the use of labeled retinol. Vitam Horm. 1974;32:251–75.

16. Matthews D, Hosker J, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.

17. V Diretrizes Brasileiras de Hipertensão Arterial 2006; Sociedade Brasileira de Cardiologia (SBC); Sociedade Brasileira de Hipertensão (SBH); Sociedade Brasileira de Nefrologia. São Paulo; 2006.

18. American Diabetes Association. Standards of medical care in diabetes. Diabetes Care 2005;28:S4–36.

19. Crespo J, Fernández-Gil P, Hernández-Guerra M, et al. Are there predictive factors of severe liver fibrosis in morbidly obese patients with non-alcoholic steatohepatitis? Obes Surg. 2001;11:254–7.

20. Papadia FS, Marinari GM, Camerini G, et al. Liver damage in severely obese patients: a clinical–biochemical–morphologic study on 1,000 liver biopsies. Obes Surg. 2004;14:952–8.

21. Garcia-Monzon C, Martin-Perez E, Lacono OL, et al. Characterization of pathogenic and prognostic factors of nonalcoholic steatohepatitis associated with obesity. J Hepatol. 2000;33:716–24.

22. Mottin CC, Moretto M, Padoin AV, et al. The role of ultrasound in the diagnosis of hepatic steatosis in morbidly obese patients. Obes Surg. 2004;14:635–7.

23. Wolf AM, Busch B, Kuhlmann HW, et al. Histological changes in the liver of morbidly obese patients: correlation with metabolic parameters. Obes Surg. 2005;15:228–37.

24. Arun J, Clements RH, Lazenby AJ, et al. The prevalence of nonalcoholic steatohepatitis is greater in morbidly obese men compared to women. Obes Surg. 2006;16:1351–8.

25. Sarni RS, Pitta TS, Fernández AP, et al. Avaliação da peroxidação lipídica e nível sérico de retinol e carotenóides em crianças e adolescentes matriculados em escola pública do município de Santo André, São Paulo. RAMB 2002;48:48–53.

26. Mecocci P, Polidori MC, Troiano L, et al. Plasma antioxidants and longevity: a study on healthy centenarians. Free Radic Biol Med. 2000;28:1243–8.

27. Gopal DV, Rosen HR. Abnormal findings on liver function tests. Postgrad Med. 2000;107:100–14.

28. Rocchi E, Borghi A, Paolillo F, et al. Carotenoids and liposoluble vitamins in liver cirrhosis. J Lab Clin Med. 1991;118:176–85.

29. Newsome PN, Beldon I, Moussa Y, et al. Low serum retinol levels are associated with hepatocellular carcinoma in patients with chronic liver disease. Aliment Pharmacol Ther. 2000;14:1295–301.

30. Romagnuolo J, Andrews C, Bain VG, et al. Simple clinical variables predict liver histology in hepatitis C: prospective validation of a clinical prediction model. Scand J Gastroenterol. 2005;40:1365–71.

31. Peres, WA. Relação entre o estado nutricional de vitamina a, estágios da doença e parâmetros bioquímicos de função e lesão hepática em pacientes com hepatite crônica, cirrose hepática e carcinoma hepatocelular pelo virus c. Rio de Janeiro, 2006. Dissertação (Doutorado). Universidade Federal do Rio de Janeiro.

32. Santos HS, Cruz WM. A terapia nutricional com vitaminas oxidantes e o tratamento quimioterápico oncológico. Rev Bras Cancerol. 2001;47:303–8.

33. Sigiura M, Nakamura M, Ikoma Y, et al. The homeostasis model assessment-insulin resistance index is inversely associated with serum carotenoids in non-diabetic subjects. J Epidemiol. 2006; 16:71–8.

34. Paolisso G, Giugliano D. Oxidative stress and insulin action: is there a relationship? Diabetologia 1996;39:357–63.

35. Facchini FS, Humphreys MH, DoNascimento CA, et al. Relation between insulin resistance and plasma concentrations of lipid hydroperoxides, carotenoids, and tocopherols. Am J Clin Nutr. 2000;72:776–9.

36. Ford ES, Will JC, Bowman BA, et al. Diabetes mellitus and serum carotenoids: findings from the Third National Health and Nutrition Examination Survey. Am J Epidemiol. 1999;149:168–76.

37. Viroonudomphol D, Pongpaew P, Tungtrongchitr R, et al. The relationships between anthropometric measurements, serum vitamin A and E concentrations and lipid profiles in overweight and obese subjects. Asia Pac J Clin Nutr. 2003;12:73–9.