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Original Article

RELATIONSHIP OF THE NUTRITIONAL STATUS OF VITAMIN A AND THE REGRESSION OF HEPATIC STEATOSIS AFTER ROUX-EN-Y GASTRIC BYPASS SURGERY FOR TREATMENT OF CLASS III OBESITY

Relação entre o estado nutricional de vitamina a e a regressão da esteatose hepática após gastroplastia em Y-de-Roux para tratamento da obesidade classe III

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ABSTRACT - ***Background*** – Vitamin A participates in several essentials functions in the human body and their serum concentrations may be decreased in non-transmissible diseases. ***Aim*** - To assess the relationship of the nutritional status of Vitamin A through the serum concentrations of retinol and beta carotene, with regression of hepatic steatosis in individuals who undergone Roux-en-Y gastric bypass surgery for treatment of class III obesity. ***Methods*** - Were included 30 individuals, male and female, submitted to Roux-en-Y gastric bypass for treatment of class III obesity, who were diagnosed through an abdominal ultrasonography as presenting hepatic steatosis. From the result of an ultrasonography screened six months after the surgical procedure those subjects were divided into two groups: group 1 – patients with steatosis detected in the ultrasonography (16 subjects) and group 2 – patients without steatosis detected in the ultrasonography (14 subjects). Before and six months after the surgery, were carried out anthropometrical analyses and biochemical exams (basal insulin, glicemy, Homeostasis Model Assessment Index (HOMA IR), cholesterol, HDL, LDL, triglycerides, AST, ALT, Gamma-GT, albumin, total bilirubin, retinol, and beta carotene. ***Results*** - The individuals presented an average weight loss of 35.05 + 10.47 ($p<0.01$) and a decrease in the Body Mass Index (BMI) of 11.6 + 4.99 Kg/m^2 ($p<0.01$). After six months, all the biochemical exams presented a significant decrease in their basal concentrations ($p<0.05$). In the post-operative period the group 2 (without steatosis) presented concentrations significantly lower ($p<0.05$) in the following variables: weight, BMI, AST, ALT, Gamma-GT, HOMA IR, basal insulin. The albumin serum concentrations presented close average values, with no significant difference in the two periods evaluated. In the assessment of retinol and beta carotene, higher serum concentrations and a small decrease in relation to the concentrations of the pre-operative period in the group without steatosis were observed, however, no statistical difference was found. ***Conclusion*** – An adequate nutritional status of vitamin A might contribute in the improvement of the hepatic esteatosis after Roux-en-Y gastric bypass for class III obesity treatment.

HEADINGS – Nutritional status. Vitamin A. Fatty liver. Gastroplasty. Therapeutics. Obesity, morbid

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RESUMO - ***Racional*** – A vitamina A participa de várias funções primordiais no organismo humano e as suas concentrações séricas podem estar diminuídas nas doenças crônicas não transmissíveis. ***Objetivo*** - Avaliar a relação entre o estado nutricional da vitamina A, e a regressão da esteatose hepática em indivíduos submetidos à gastroplastia em Y-de-Roux para tratamento da obesidade classe III. ***Métodos*** - Foram estudados 30 pacientes obesos classe III, de ambos os sexos, com esteatose hepática, submetidos à gastroplastia em Y-de-Roux. Seis meses após a operação, os pacientes foram submetidos à ultrassonografia abdominal e distribuídos em dois grupos: grupo 1 - pacientes com esteatose detectada na ultrassonografia e grupo 2 – pacientes sem esteatose detectada na ultrassonografia. No pré-operatório e seis meses após a operação foram realizadas análises antropométricas e exames bioquímicos: insulina basal, glicemia, Homeostasis Model Assessment Index (HOMA IR), colesterol, HDL, LDL, triglicerídeos, AST, ALT, Gama-GT, albumina, bilirrubina total, retinol, e beta caroteno. ***Resultados*** – A média de perda de peso foi de 35,05 + 10,47 ($p<0,01$) e a diminuição no Índice de Massa Corporal (IMC) de 11,6 + 4,99 Kg/m^2 ($p<0,01$). Após seis meses, todos os exames bioquímicos apresentaram diminuição

significativa das concentrações (p<0,05). O grupo 2 (sem esteatose) apresentou no pós-operatório, concentrações significativamente inferiores (p<0,05), nas variáveis: peso, IMC, AST, ALT, gama GT, HOMA IR, insulina basal. As concentrações séricas de albumina apresentaram valores médios aproximados, sem diferença significativa nos dois tempos avaliados. Observaram-se concentrações séricas mais elevadas de retinol e de β-caroteno e redução menor em relação as concentrações do pré-operatório nos pacientes sem esteatose, porém sem diferença estatística. ***Conclusão*** – Adequado estado nutricional de vitamina A pode contribuir na regressão da esteatose hepática em pacientes submetidos à Gastroplastia em Y-de-Roux para tratamento da obesidade classe III.

DESCRITORES - Estado nutricional. Vitamina A. Fígado gorduroso. Gastroplastia. Terapêutica. Obesidade mórbida.

INTRODUCTION

Obese patients present a high prevalence of diabetes mellitus type 2 (DM2), hypertension and dyslipidemia, among other diseases[24]. The nonalcoholic fatty liver disease (NAFLD), a condition often associated with obesity, presents a broad spectrum of liver injury that may vary from simple steatosis to steatohepatitis, advanced fibrosis and cirrose[1]. This disease occurs in 3-24% of the general population and is extremely common among patients undergoing bariatric surgery, ranging from 84% to 96%[5].

Vitamin A participates in several essential functions in the human body, such as visual acuity, immune activity, proliferation and differentiation celular[12]. Although pregnant women, nursing mothers, infants, toddlers and preschoolers constitute the classical vulnerable group of vitamin A deficiency, other studies indicates serum vitamin A decreased in individuals with diseases involving the absorption of lipids and in metabolic disorders, such as liver disease and diabetes mellitus type[9,28]. Recently, this vitamin as well as their precursors, was highlighted by his performance against free radicals, protecting the body against oxidative stress and thus preventing tissue damage and injuries related to various chronic non-transmissible diseases[7].

The vitamin A deficiency may be present in individuals with NAFLD, contributing to the disease, since these individuals are more susceptible to oxidative stress which, by itself, greatly increases the consumption of substances having antioxidant function[27].

Observing the results and efficiency of surgical treatment of class III obesity to reduce the complications of this disease[3,6], including hepatic steatosis[23], it was proposed to evaluate the relationship between nutritional status of vitamin A, by serum retinol and β-carotene, and the regression of hepatic steatosis in patients undergoing Roux-en-Y gastric bypass in (RYGB) for the treatment of class III obesity.

METHODS

Were evaluated 30 obese individuals, male and female, diagnosed with NAFLD undergoing RYGB for treatment of class III obesity. Inclusion criteria were: indication for surgical treatment of class III obesity (BMI> 40 kg / m^2 or BMI> 35 kg / m^2 with co-morbidities); diagnosis of hepatic steatosis performed by abdominal ultrasonography preoperatively; anthropometric assessments by the medical team before and six months after surgery; examinations and ultrasonography (USG) before and six months after surgery.

The inclusion of each patient in the study was made upon formal authorization by signing a consent form, after about the objectives and procedures of the project by the researcher, according to the standards set by the National Health Council (1987). Patients were informed that, in return for their participation in the study, they would have nutritional diagnosis, treatment of nutritional deficiencies diagnosed, individualized nutrition education and their results would be reported to them. All patients were instructed to use multivitamins with 5000 IU daily dose of retinol acetate, after the operation. The surgical team was the same in all procedures.

Exclusion criteria were: refusal to participate in the study, presence of disabsorptive syndromes, acute and chronic infections, pregnant and lactating women; associated endocrinopathies, alcohol consumption greater than 20 g / day; alcoholic liver disease, viral hepatitis, autoimmune hepatitis-immune, primary biliary cirrhosis, primary sclerosing cholangitis, liver metabolic diseases and drug-induced hepatitis or other liver disease other than NAFLD, have made prior bariatric surgery.

Were analyzed the following variables: weight, height and body mass index (BMI), insulin resistance determined by the Homeostasis Model Assessment Index (HOMA)[12]; plasma basal insulin expressed in mcu / ml, plasma levels of fasting glucose in mg / dl; lipid profile evaluated by serum levels of total cholesterol, triglyceride, high density lipoprotein (HDL) and low density lipoprotein (LDL), expressed in mg / dL; function tests and lesion liver by serum levels of albumin (g / dL), total bilirubin (g / dL) and gamma-glutamyl transpeptidase (GGT) (U / L) and AST (U / L) ALT (U / L) ; nutritional status of vitamin A, by the determination of serum retinol and

β-carotene, using HPLC (High Performance Liquid Chromatography) method and expressed in mmol / L and mg / dL, respectively.

The surgical procedure performed in these patients was RYGB. This consists of making a small gastric pouch vertical, with reconstruction of the Roux-en-Y. The gastrojejunal anastomosis was manual and calibrated (0.8 - 1.2 cm) and jejunojejunal anastomosis was made 100-150 cm from the former. The operation was conventional or laparoscopic[13].

Two analyzes were performed: an overall assessment, noting the effect of the operation in the total group (30 patients), comparing the variables of the postoperative (six months) with those of the preoperative.

The other analysis was made from the distribution of subjects into two groups: group 1 - those with steatosis and group 2 - without steatosis, from the USG performed postoperatively. This way, it was possible to identify, whether there was any influence of the nutritional status of vitamin A in the patients who improved the degree of liver disease.

For data analysis were used nonparametric tests (Wilcoxon and Mann-Whitney test) for variables not normally distributed. For those who have normal distribution, was used the Student t test. The test of normality was performed using the Kolmogorov-Smirnov test. The significance level was set at 0.05. This study was approved by the Ethics and Research of the University Hospital Clementino Fraga Filho (CAE 007.0.197197-06).

RESULTS

Overall rating

Among those studied, 19 (63.3%) were female and 11 (36.7%) male. The average age of the group was 43.15 ± 11.36 years, ranging from 19 to 60. Except for HDL and albumin, all variables showed a significant decrease (p <0.05), six months after the operation (Table 1). After the postoperatively ultrasound were identified 16 individuals (53.3%) maintaining hepatic steatosis and 14 (46.6%) no longer had the disease characteristics.

Evaluation between groups

Postoperative

From the results obtained in each group, six months after the operation, it was observed that 11 patients without hepatic steatosis were female and three male. Among the individuals in the group 1, there were eight men and eight women. The analysis of the mean age of the groups showed that patients without hepatic steatosis (40.29 ± 11.77) were younger than patients with steatosis (46.44 ± 10.51).

TABLE 1 - Variables studied before and six months after RYGB (Overall)

	Preoperative	Postoperative (6 meses)	p
Weight (Kg)	129,9 + 23,1	94,95 + 24,46	**<0,01***
BMI (Kg/m^2)	45,9 + 5.63	34,31 + 8.17	**<0,01***
Glucose (mg/dL)	114,4 + 37,3	89,6 + 13.1	**0,002****
HOMA IR	5.95 + 2.78	2,44 + 1,57	**<0,01****
Insulin (µU/mL)	23,67 + 10,9	9,74 + 5,46	**<0,01****
Cholesterol (mg/dL)	195 + 35,81	162,53 + 31,93	**<0,01***
HDL (mg/dL)	46,31 + 10,6	43,6 + 9,72	**0,182***
LDL (mg/dL)	113,41 + 27,6	92,66 + 23,29	**<0,01***
Triglycerides (mg/dl)	175,63 + 10,34	111,33 + 34,92	**<0,01****
AST (U/L)	32,58 + 13,24	23,1 + 10,39	**0,01****
ALT (U/L)	45,17 + 20	24,2 + 14,8	**<0,01****
GGT (U/L)	44,11 + 23,89	18,7 + 14,9	**<0,01****
BT (mg/dL)	0,61 ± 0,14	0,8 ± 0,18	**<0,01****
Albumin (g/dL)	4,02 + 0,43	4,18 + 0,33	**0,09****
Retinol (µmol/L)	1,69 + 0,55	1,34 + 0,57	**0,019***
B-carotene (µg/ dl)	68,84 + 48	33,8 + 23,39	**<0,01****

Bold = significant result
BT = total bilirubin
* t test
** Wilcoxon test

The group 2 showed significantly reduction of weight (p = 0.048), BMI (p = 0.01), IR (HOMA-IR p = 0.019), basal insulin (p = 0.02) and liver enzymes (AST p = 0.012, p = .02 ALT, GGT p = 0.01) (Table 2).

TABLE 2 - Comparison between groups 1 and 2 six months after the operation

	GROUP 1 WITH STEATOSIS	GROUP 2 WITHOUT STEATOSIS	p
Weight (Kg)	103,91 + 26,17	84,71 + 18,26	**0,048***
BMI (Kg/m^2)	37,85 + 8,25	30,28 + 6.14	**0,011***
Glucose (mg/dL)	89,25 + 10,24	90 + 16.06	0,917*
HOMA IR	2.97 + 1,86	1,85 + 0,89	**0,049****
Insulin (µU/mL)	11,8 + 6,25	7,41+ 6,23	**0,022****
Cholesterol (mg/dL)	162,25 + 28,6	162,86 + 36,48	0,96*
HDL (mg/dL)	41,56 + 8,63	45,93 + 10,69	0,226*
LDL (mg/dL)	97,31 + 18,82	87,36 + 27,29	0,25*
Triglycerides (mg/dl)	111,19 + 27,67	111,50 + 42,87	0,982*
AST (U/L)	26,81 + 10,89	18,86 + 8,23	**0,012****
ALT (U/L)	29,94 + 15,89	17,64 + 10,67	**0,02****
GGT (U/L)	25,56 + 17,49	10,86 + 4,28	**0,01****
BT (mg/dL)	0,69 ± 0,18	0,65 ± 0,19	0,692**
Albumin (g/dL)	4,21 + 0,35	4,15 + 0,31	0,651*

Bold = significant result
BT = total bilirubin
* t test
** Wilcoxon test

The postoperatively lipid profile analysis showed no significant difference between the two groups. Likewise, blood glucose and albumin had to be quite similar (Table 2).

Variation between preoperative and postoperative

There was a decrease in the postoperatively results compared with those of the preoperative, most pronounced in the group 2 variables: weight, BMI, AST, ALT, cholesterol, LDL, triglycerides - however, without statistical significance (Table 3).

TABLE 3 - Comparison of the variation between pre and postoperative groups 1 and 2

	GROUP 1 WITH STEATOSIS	GROUP 2 WITHOUT STEATOSIS	p
Weight (Kg)	33,71 ± 11,41	37,03 ± 9,29	**0,29***
BMI (Kg/m^2)	9,9 ± 4,69	13,53 ± 4,77	**0,06***
Glucose (mg/dL)	28,13 ± 38,61	21,00	0,636**
HOMA IR	3,77 ± 3,01	3,43 ± 3,03	**0,774***
Insulin (µU/mL)	15,85 ± 10,81	11,72 ± 7,92	**0,262****
Cholesterol (mg/dL)	28,4 ± 34,61	36,36 ± 25,87	0,492*
HDL (mg/dL)	3,33 ± 7,51	1,14 ± 9,24	0,488*
LDL (mg/dL)	13,33 ± 24,4	29 ± 23,4	0,087*
Triglycerides (mg/dl)	49,88 ± 48,79	80,79 ± 106,78	0,306*
AST (U/L)	8,2 ± 11,29	12,36 ± 16,96	**0,431***
ALT (U/L)	19,87 ± 21,07	24,43 ± 24,11	**0,591***
GGT (U/L)	27,78 ± 32,14	26,54 ± 18,69	**0,847***
BT (mg/dL)	0,22 ± 0,21	0,13 ± 0,25	0,618**
Albumin (g/dL)	-0,23 ± 0,44	-0,04 ± 0,41	0,223*

Bold = significant result
BT = total bilirubin
* t test
** Wilcoxon test

Results of the levels of retinol and β-carotene

It was observed that the average postoperative serum retinol was higher in the group 2 (1.50 + 0.56 mmol / l) than in group 1 (1.21 + 0.57 micromol / l), although it was not statistically significant (p = 0.171). Similarly, there was a less pronounced reduction of retinol in these individuals, from the results of preoperative (p = 0.398) (Figure 1).

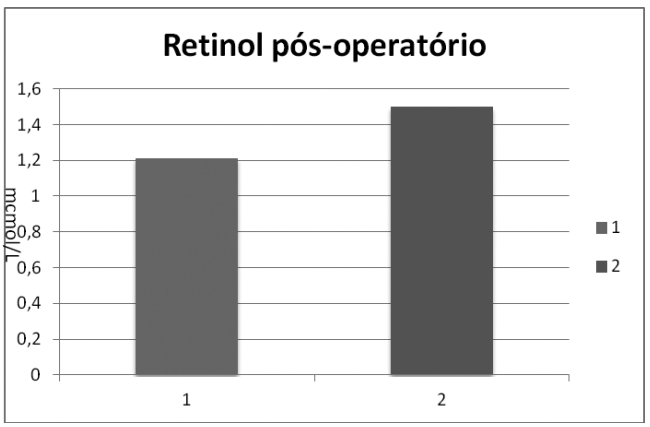

FIGURE 1 - Serum retinol six months after operation in groups 1 and 2 p= 0,171 (t test)

The behavior of serum β-carotene six months after the operation was similar to that of retinol, with a higher mean serum levels and a less pronounced variation between pre and postoperative in the group 2. These results were not considered significant (Figures 2 and 3).

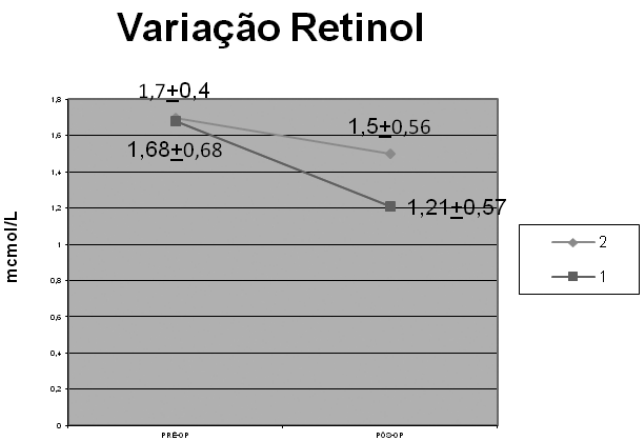

FIGURE 2 - Variation of serum retinol between pre and postoperative periods. p = 0.398 (t test).

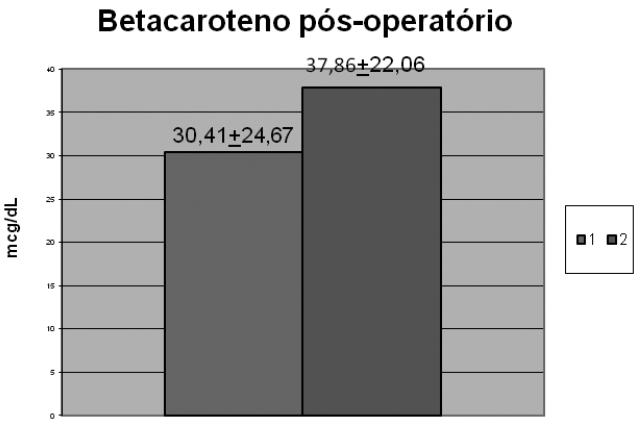

FIGURE 3 - Serum β-carotene six months after operation in groups 1 and 2. p = 0.219. (Mann-Whitney)

DISCUSSION

Overall rating

Surgical treatment is the most effective for patients with class III obesity[6]. This study observed an average weight loss of 35.05 ± 10.47 kg (26.98%) and a decrease in the BMI of 11.6 ± 4.99 kg / m² in the first six months after the operation. This result can be considered satisfactory, since there was a significant improvement in several metabolic parameters, such as IR, lipid profile and liver enzymes.

Buchwald et al.[14] observed after RYGB, resolution of DM2 in 78.1% of patients and improvement and resolution in 86.6%. Regarding dyslipidemia, the same author found, evaluating various models of bariatric surgery, a decrease of 33.20 mg / dL in serum total cholesterol, 29.34 mg / dL in LDL and 79.65 mg / dL in triglyceride[15]. These results were similar to those found in our study where there was a decrease of 32.24, 20.79 and 64.30 mg / dL, respectively, in serum total cholesterol, LDL and triglycerides (p <0.01).

The beneficial effect of bariatric surgery on NAFLD, by reducing the weight and improving metabolic

syndrome, has been already described[16,17,18]. Mattar et al followed 70 patients who underwent bariatric surgery with a diagnosis of NAFLD confirmed by liver biopsy and detected resolution of hepatic steatosis in 37% of patients and fibrosis in 20%. There was significant reduction in weight, BMI, blood glucose, total cholesterol, triglycerides, LDL, AST and ALT[19].

This analysis did not detect hepatic steatosis on ultrasonography in 46.6% of subjects studied six months after the operation, but to confirm the disappearance of steatosis, there would need a liver biopsy. However, was observed a significant improvement in several metabolic parameters considered risk factors for NAFLD. This fact may have contributed to the regression of hepatic steatosis in these patients.

Comparison between groups 1 and 2

From the results obtained postoperatively, there was a difference in the profile of the groups. Patients which the USG did not detect liver steatosis had lower weight and BMI, serum levels of HOMA-IR, basal insulin and liver enzymes significantly lower. With this, one can distinguish this group of lower weight, smaller degree of insulin resistance and reduced serum markers of liver injury. These data show the characteristics of patients with NAFLD. Marchesini et al.[20], using the HOMA, proved insulin resistance was the most directly related laboratory finding with NAFLD. The strong association of this disease with obesity, central fat distribution, DM2, dyslipidemia, hypertension and atherosclerotic disease, supports the hypothesis that NAFLD may represent a further condition of metabolic syndrome[21].

In a study with 1022 Korean trying to correlate metabolic syndrome with the severity of hepatic steatosis (graded through sonographic criteria), it was found that as the degree of steatosis increased, there was a raise in weight, IR (measured by HOMA), serum levels of liver enzymes and also in serum cholesterol and triglycerides[22].

According to some studies evaluating the epidemiology of NAFLD, this condition is more common in men and its incidence increases with age[3,23]. This fact is consistent with the finding of our analysis where the predominance of females was higher in the group 2 (11/14). Among the subjects with hepatic steatosis, this ratio decreased because men and women were found in the same proportion. Regarding age, group 2 patients had a lower mean age (40.29 ± 11.77) compared to those of group 1 (46.44 ± 10.51), however, there was no statistical significance in this analysis ($p > 0.05$).

When the variation between pre and postoperative is analyzed, it can be observed that the operation caused changes very similar in both groups. Thus, it is evident that the RYGB was effective for all individuals, and some, probably because they had risk factors for NAFLD as obesity, RI, dyslipidemia and oxidative stress, more intense in the preoperative, they still maintained degree of liver disease somewhat more advanced at the time when the analysis was done.

Assessment of nutritional status of vitamin A after operation

After RYGB was observed a significant decrease in the levels of β-carotene and retinol. This may be justified because vitamin A is fat soluble and 24 after the operation lipid absorption is decreased due to reduced intake and jejunal bypass. Moreover, the ingestion of foods rich in Vitamin A is also reduced. The vitamin A deficiency after RYGB has been reported previously25 and there are described cases of night blindness and xerophthalmia [26].

This study demonstrates that the decrease in serum β-carotene after the operation, is higher compared to the levels of retinol. It is known that β-carotene is the most potent precursor of retinol, and may be converted, according to the bioavailability of retinol[12]. Therefore, this high reduction of β-carotene concentrations can be justified by its conversion to retinol. Group 1 individuals demonstrated lower serum retinol and β-carotene concentrations. Although with no statistical significance, this finding suggests that vitamin A may be associated with regression of hepatic steatosis after RYGB.

The excessive deposition of fatty acids in the liver causes an increase in oxidative stress, which may be responsible for progression of hepatic steatosis to non alcoholic steatohepatitis (NASH) and subsequently to cirrhosis[2]. The use of antioxidants such as vitamins C and E in the treatment of NAFLD, produces satisfactory results resulting in regression of inflammation and fibrosis[27].

The retinol and carotenoids are efficient free radical scavengers, which protect the body against oxidative stress and, consequently, cell injury. Therefore, as the patients with NAFLD have elevated levels of lipid peroxidation, they can have a increased use of antioxidants, including vitamin A.

After surgical treatment, due to the weight loss and improvement of co-morbidities there is a reduction in oxidative stress. Lipid peroxidation, measured by plasma levels of malondialdehyde, decreases significantly12 and 24 weeks after vertical banded gastroplasty and serum α-tocopherol increases significantly 24 weeks after the operation, since the levels of β-carotene present non significant increase[28].

Therefore, it is expected after RYGB, a reduction of lipid peroxidation, with consequent improvement of NAFLD and increase of antioxidants concentrations.

Although the mean serum retinol and β-carotene was reduced in group 1 patients, there was no evidence of this relationship through the statistical analysis used in the present analysis. One explanation for this result may be the small number of subjects studied (30 patients). Perhaps, in a study using a larger sample, this relationship can become significant.

The reports in the literature of the relationship

between serum vitamin A and NAFLD are scarce. In a study using an experimental model of transgenic mice with liver retinoic acid receptors defective, was observed the development of NASH with four months of life and hepatocarcinoma at 12 months. Animals that received a diet rich in retinoic acid since three weeks of age did not develop histological changes in the liver. These facts suggest a protective effect of retinoic acid in the development of NAFLD and hepatocarcinoma [29].

Rocchi et al., measured the concentration of fat soluble antioxidant vitamins in patients with hepatic cirrhosis compared to healthy controls and found significantly reduced concentrations of retinol and other fat-soluble vitamins in these patients[30].

Yadav et al. observed that the concentrations of retinol and other antioxidants were markedly depleted in patients with liver disease, whereas the worsening of fibrosis was associated with a decrease of retinol and other antioxidants in the liver, which may be a consequence of antioxidant depletion or decreased liver stock due to fibrosis[31].

These findings are different from those found in this study, where the aim was to detect whether the antioxidant effect of vitamin A would be evident with the regression of hepatic steatosis occurred after surgical treatment. Despite not having been proven statistically, it was observed that the decrease in mean serum retinol in relation to preoperative was lower in patients with a greater regression of steatosis. When the same variation is analyzed in relation to β-carotene, was identify a fall much less pronounced in these patients. This is important, since the β-carotene has an antioxidant effect more intense with respect to retinol and may have facilitated the regression of hepatic steatosis in this group of patients.

As discussed above, several studies show the relationship between the severity of liver disease with low serum vitamin A. However, publications relating vitamin A with NAFLD are scarce and conflicting, characterizing this relationship as an open field for further research.

CONCLUSION

Was found that there was a distinction between the two groups. Patients who did not keep signs of hepatic steatosis on ultrasound six months after the procedure, presented, in relation to other individuals, metabolic changes consistent with a lower concentration of oxidative stress, higher serum levels of retinol and β-carotene in postoperative and a smaller drop in these levels relative to preoperative. Although these results were not confirmed by statistical analysis employed, individuals who maintained hepatic steatosis probably had a higher consumption of antioxidants such as β-carotene and retinol. Thus, one can infer that a good nutritional status of vitamin A may contribute to regression of liver steatosis in patients undergoing Roux-en-Y Gastric Bypass for treatment of class III obesity.

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