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Original

Nutritional supplementation assessment with whey proteins and TGF-β in patients with Crohn's disease

T. Davanço[1], V. Oya[1], C. Saddy Rodrigues Coy[2], R. Franco Leal[2], M.ª de L. Setsuko Ayrizono[2], V. C. Sgarbieri[3], M.ª M. dos Santos Vilela[1] and E. A. Lomazi[1]

[1]Centre for Research in Pediatrics and Pediatrics Department.School of Medical Sciences. UNICAMP. [2]Surgery Department. School of Medical Sciences. UNICAMP. [3]Department of Food and Nutrition. School of Food Engineering. UNICAMP. Brazil.

Abstract

Crohn's disease (CD) is a chronic inflammatory disorder that primarily affects the intestines, resulting in breakage of the intestinal barrier, pathological inflammation and nutritional disorders that encompass from trace elements deficiency to severe malnutrition. Nutritional interventions either alone or associated to drug therapy may be effective to achieve and maintain inflammation remission.

***Objective:* To evaluate usual food intake as quantitative and qualitatively, in CD patients; and describe the effect of a supplement containing whey proteins and TGF- on their body composition.**

***Patients and methods:* Dietary intake was assessed considering 42 consecutive patients, followed in a tertiary center, and by using the 3-day food recall and food intake frequency questionnaire. Body composition was assessed previously and 8 weeks after supplementation with a diet containing whey proteins and TGF-β (N = 22).**

***Results and discussion:* Considering carbohydrates and lipids, most patients had adequate dietary intake according recommendations. Protein, saturated fat, B12 vitamin and zinc intakes were higher than the recommended values. The dietary fiber, A, D, C and E vitamins, calcium, iron, folate, potassium and sodium intakes did not reach the recommended requirements in most patients. Patients supplemented with the whey protein and TGF-β dietary presented a positive increment in their lean body mass, when compared to non-supplemented group.**

***Conclusion:* CD patients require nutritional orientation. Whey protein intake resulted in significant differences, such as improvement in Lean Body Mass and reduction in Fat percentage.**

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Key words: *Chron's disease. Nutritional assessment. Food consumption. Food intake. Body composition. Supplementation.*

Correspondence: Elizete Aparecida Lomazi.
University of Campinas – Unicamp.
Brazil.
E-mail: elizete.apl@gmail.com

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EVALUACIÓN DEL EFECTO DE LA SUPLEMENTACIÓN CON PROTEÍNAS DEL SUERO DE LA LECHE Y EL TGF-β, EN PACIENTES CON SÍNDROME DE CROHN

Resumen

La enfermedad de Crohn (EC) es un desorden inflamatorio crónico, que afecta principalmente el intestino, resultando en el rompimiento de la barrera intestinal, inflamación patológica y disturbios nutricionales que pueden variar de la deficiencia de oligoelementos a la desnutrición grave. Intervenciones nutricionales pueden ser eficaces para alcanzar y mantener la remisión del proceso inflamatorio, aisladamente, o en combinación con terapia farmacológica.

***Objetivo:* Evaluar el consumo alimentario cuantitativo y cualitativo en pacientes con EC y el efecto de la suplementación nutricional con dieta conteniendo proteínas del suero de leche y TGF-β, en la composición corporal de los pacientes.**

***Pacientes y métodos:* La ingestión alimentaria fue evaluada en 42 pacientes, aplicando el registro alimentario de 3 días y el cuestionario de frecuencia alimentaria. La composición corporal fue evaluada en esos pacientes, siendo que 22 recibieron la dieta conteniendo proteínas del suero de leche y TGF-β y 20 pacientes no fueron suplementados (grupo control).**

***Resultados y discusión:* La mayoría de los pacientes presentó ingestión alimentaria adecuada de carbohidratos y lípidos. El consumo de proteína, grasa saturada, vitamina B12 y zinc fue superior a los valores recomendados. El consumo de fibras alimentarias, vitamina A, D, C, y E, calcio, hierro, folato, potasio y sodio no satisfizo las necesidades recomendadas en la mayoría de los pacientes. Hubo un aumento de la masa magra de los pacientes suplementados con la proteína del suero de leche y TGF-β.**

***Conclusión:* El grupo de pacientes con enfermedad de Crohn presentó, en su mayoría, un consumo adecuado de carbohidratos y lípidos, y el consumo de proteína fue superior al recomendado en más de la mitad de los pacientes. Se encontró una ingesta inadecuada de vitaminas A, C, D y E, fibras, calcio, folato, hiero y zinc. La ingestión de proteína del suero de leche mejoró la composición corporal de los pacientes.**

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Palabras clave: *Enfermedad de Crohn. Evaluación nutricional. Consumo alimentario. Ingesta de alimentos. Composición corporal. Suplementación.*

Abbreviations

CD: Crohn's Disease.
GSH: Glutathione.
TEV: Total energy value.
BMR: Basal metabolic rate.
BMI: Body mass index.

Introduction

Over the years, nutritional therapy has taken an increasingly role in CD therapy, exerting a nutritional restorative action over body compartments, and minimizing catabolic effects caused by the disease. The importance of food was enhanced by the ability of inducing inflammatory remission by exclusive ingestion of elemental formulas, which reduces the stimulus of food antigens. More currently, the nutritional focus on CD has been the immunomodulatory capacity of some specific nutritional elements that act by modulating the immunoinflammatory response and by maintaining the integrity of intestinal mucosa.[1]

Thus, a rational nutritional therapeutic plan to CD patients should include nutrients to supply calories, reduce food antigenic stimulation, regulate inflammatory and immune response, and stimulate the mucosal tropism.[2]

Whey concentrate contains significant amounts of cysteine and glutamylcysteine amino acids, glutathione forerunners (GSH), and the mixture of these proteins plays an important role in maintaining cellular levels of GSH.[3] Further, whey proteins showed specific physiological actions and are associated with many biological effects, such as anticancer activity, improved digestive function, improved physical performance and immune system modulation.[4,5,6,7] So, it seems to be worth to evaluate this therapy effects on CD patients as being followed in a Brazilian specialized center for IBD.

The objectives of this study were to evaluate food intake in Crohn's disease patients and the effect of whey proteins and TGF- on body composition.

Materials and methods

Prospective study based on clinical and nutritional trial intervention with a whey concentrate enriched with TGF-β. It was approved by the Independent Ethics Committee of FCM-UNICAMP (doc. Nº 304/2007) and all participants signed an informed consent form.

Subjects

Patients were recruited into a tertiary out clinic specialized in IBD at the UNICAMP School Hospital. Patients who agreed to participate were included when according with the following inclusion criteria: 1. Patients of both genders; 2. Prescribed with biological therapy: anti-TNF-α, 5-10 mg/kg, every 2 months (REMICADE®-Mantecorp-Brazil) and used in combination with azathioprine; 3. Small bowel inflammatory involvement was established on colonoscopy and biopsy; 4. No smoking or drug or other medication use; and 5. Have a previous history of regular adherence to outpatient follow-up, according to service records.

Nutritional assessments were performed in three stages, in the group of patients who received supplementation. Times were set according to the following:

- T0: immediately before anti-TNF-α infusion.
- T1: 8 weeks after T0, immediately before anti-TNF-α infusion.
- T2: 8 weeks after T1.

Those patients who were eligible to study but who did not agree on supplementation trial were assessed in two stages:

- T0: immediately before anti-TNF-α infusion.
- T2: 16 weeks after T0.

Supplementation product contains whey protein concentrate plus TGF-β, the prescribed doses consisted of 50% of Recommended Dietary Allowances for proteins in whey protein concentrate.[8] The concentrate was donated by Hilmar Cheese Company, 9001 (North Lander Avenue Hilmar, California 95324 USA).

Data reported at this paper comes from a prospective and interventional study whit the main objective was to investigate the effect of a whey protein concentrate containing TGF-β, administered for 16 weeks, on the inflammatory response of Crohn's disease, with focus in oxidant system vs. the antioxidant system, so, authors did not define a specific nutritional status as supplementation criteria for including subjects (table I).

Table I
Composition characteristics of whey protein supplement are presented in table below

Whey proteins supplement composition	
Source	*Supplement*
Protein (%)[1,2]	73.30 ± 0.43
Lipid (%)[1,2]	10.48 ± 0.03
Gray (%)[1,2]	2.15 ± 0.33
Water (%)[1,2]	6.36 ± 0.01
Carbohidrate (%)[2,3]	7.71 ± 0.01

[1]Mean from 3 dosages (± DV) de três determinações.
[2]Vallues expressed on dry basis.
[3]Value obtained as = 100 – (protein + total lipids + gray + water).

Food intake

A 3-day food record questionnaire was applied on 3 different days, individually and previously supplementation period. Usual diet was explored with regards on quantity, brand and preparation of each food. This exploration has used a photo album where patients could compare size and amount of their portions. By this method, the energy intake was estimated (total energy value-TEV); percentage of carbohydrates, proteins and lipids in relation to ingested TEV; percentage of saturated, polyunsaturated and monounsaturated fats in relation to TEV; contents of fiber, vitamin A, vitamin D, vitamin C and vitamin E, vitamin B_{12}, folate, calcium, iron, zinc, potassium and sodium. Estimative were performed using software AVANUTRI version 3.1.4, based on the Food Composition Table (TACO),[9] the Table of WHO/FAO, 2003[10] was consulted to assess the ratio adequacy of carbohydrates, proteins and lipids, and DRI[11] was consulted as a comparison parameter to assess the adequacy of micronutrients intake.

Harris and Benedict equation modified by Long et al.[12] was used to investigate the adequacy of energy intake as following:

- Men: (BMR)* = 66 + (13.7 x weight in kg) + (5 x height in cm) - (6.8 x age in years).
- Women: (BMR)* = 655 + (9.6 x weight in kg) + (1.7 x height in cm) - (4.7 x age in years).

*BMR = basal metabolic rate.

To evaluate the qualitative intake, the Food Intake Questionnaire was applied. The questionnaire was previously tested and included 135 dishes that have been classified into 11 groups: soups and pasta, meat and fish, dairy products, beans and eggs, rice and tubers, vegetables, sauces and spices, fruits, beverages, breads and biscuits, pastries and desserts.

The consumption frequency was assessed as follows: daily (when food was consumed every day, the number of times it was ingested and portion amount), weekly (when it was consumed every week, the number of times it was ingested during a week and portion amount), monthly (when it was consumed every month, the number of times that it was consumed during a month and portion amount).

Data on food frequency were processed using Microsoft Excel 2002 (10.2614.2625)-Brazil.

Body composition

The body composition was performed following the criteria listed below:

Inclusion criteria: Patients of both genders; being treated with intravenous anti-TNF-α, 5 to 10 mg/kg each 2 months (REMICADE®-Mantecorp-Brazil); Azathioprine, prescribed orally daily; small bowel involvement according with colonoscopy; no smoking; no drugs or medications use; regular compliance to outpatient follow-up.

Patients were located in two groups:

- Supplemented group (S): 22 patients who received nutritional counseling and whey protein with TGF-β during 16 weeks,
- Non-supplemented group (NS): 20 patients who received nutritional counseling but did not agree in ingesting supplement.

Biceps, triceps, subscapular and suprailiac skin fold were measured at T0, T1 and T2 with a skin fold caliper *Lange*, according to the procedure described by Durnin & Rahman, 1967.[13]

Statistical analysis

Numerical variables were compared with Friedman test and adopting a significance level lower than 0.05. Data were analyzed using SPSS for Windows 15.0 (SPSS Inc., Chicago, Illinois). Values of Food Frequency Questionary for the most consumed food were evaluated according to their daily, weekly or monthly intake frequency and data were processed using Microsoft Excel 2002 (10.2614.2625)-Brazil.

Results

In the supplemented group, 17 patients were male and median age was 37 years, ranging from 16 to 62 years old.

Table II shows the adequacy of energy and nutrients intake in 42 CD patients, before supplementation and according with their feeding habits.

Values of protein intake were found in quantities upper the recommended value, according WHO/OMS,[14] but, when classified by protein sources, patient's intake of fish, chicken and lentils was low, while meat, milk, rice, and beans were consumed in quantities above the recommended value (table III).

In relation to carbohydrates intake[10], it was found that about a half of the patients reached the recommended values, carbohydrates intake frequency was also low. Similarly, fiber intake was found below the recommended value.

Most patients referred adequate intake of monounsaturated and polyunsaturated fats, but consumption of saturated fats was above recommendations.[10]

Regarding micronutrients intake, it was found low intake of vitamins A, C, D and E, since that most fresh foods are not frequently consumed. Folate intake was also under the recommended value. Vitamin B_{12} was consumed in amounts 59% higher than the recommended value. In the present series, it was noticed low intake of calcium, iron and potassium. Intake of vita-

Table II
Intake and adequacy of energy and nutrients intake as evaluated in 42 patients with Crohn disease and classified according with reference values[12]

Nutrients	*Adequate*	*Below*	*Above*
Carbohydrates	23 (54.7%)	19 (45.2%)	–
Proteins	12 (28.6%)	13 (30.9%)	17 (40.5%)
Lipids	31 (73.8%)	3 (7.1%)	8 (19%)
Saturated Fat	8 (19%)	–	34 (81%)
Monounsaturated fat	27 (64.9%)	–	15 (35.7%)
Polyunsaturated Fat	38 (90.5%)	1 (2.3%)	3 (7.1%)
Fibers	12 (28.6%)	29 (69%)	1 (2.3%)
Vitamin A	3 (7.1%)	23 (54.8%)	15 (35.7%)
Vitamin D	3 (7.1%)	33 (78.6%)	6 (14.9%)
Vitamin C	3 (7.1%)	25 (59.5%)	14 (33.3%)
Vitamin E	4 (9.5%)	26 (61.9%)	12 (28.6%)
Vitamin B12	5 (11.9%)	12 (28.6%)	25 (59.5%)
Calcium	–	28 (66.7%)	14 (33.3%)
Iron	–	31 (73.8%)	11 (26.2%)
Folate	–	42 (100%)	–
Zinc	4 (9.5%)	17 (40.5%)	21 (50%)
K	–	42 (100%)	–
Na	6 (14.3%)	24 (57.1%)	12 (28.6%)

min D did not reach the minimum recommendation (table IV).

Discussion

The studied group of Brazilian patients with CD showed inadequate intake of protein and micronutrients. This inadequacy may contribute to worsening nutritional status in CD patients since it occurs in association to nutrients malabsorption due to surgical resections, bile salt deficiency, bacterial overgrowth, inflammatory activity and gastrointestinal narrowing. In addition, CD patients show anxiety and fear related to eating, emotional symptoms caused by experiences such as abdominal pain, swelling, nausea or diarrhea that are frequently observed.[15] This set of factors also contributes to deficits of trace elements and protein-energy denaturing[16] in CD patients.

Carbohydrates intake was found below the recommended values in 45% of patients. Food frequency data showed a relatively low intake of complex carbohydrates while simple carbohydrates consisted of the most of carbohydrate uptake. Recent studies found that fiber intake is very important in patients with CD, since chronic inflammation is a consequence of an exaggerated response to luminal agents, which trigger the pathogenic inflammatory cascade. So, fiber can be helpful with CD, since that in addition of being an energy source to colonocytes, fibers may inhibit inflammatory mediators' generation.[17] Studies "*in vivo*" showed that butyrate inhibits the formation of proinflammatory mediators such as alpha tumor necrosis factor and synthase nitric oxide enzyme activity.[18] Low intake of fibers occurs not only in patients with CD, but also in the healthy Brazilian population.[19] Brazilian epidemiological data (ENDEF-1974/759)[20] showed that the diet of the population from three main cities in Brazil-Rio de Janeiro, Sao Paulo and Porto Alegre - contains low quantities of dietary fiber. Thus, the poor fiber intake found in patients with CD probably reflects Brazilian population food habits.

Most patients showed adequate lipids intake, with an adequate consumption of monounsaturated and polyunsaturated fats, but the consumption of saturated fat was above the recommended value. Omega-3 and Omega-6 are metabolized into anti-inflammatory compounds, such as leukotriene in the intestine, and may have beneficial effect on carriers of intestinal inflammation.[21]

A, C, D and E vitamins intake was lower than the recommended value, as patients reported low intake of foods rich in these vitamins (table III). The Food Intake Questionnaire (QFA) is considered as the most practical and informative method for assessing the dietary intake, and is fundamentally important in epidemiological studies that correlate diet to chronic diseases.[22]

CD patients showed a protein intake above recommended requirements,[14] this study design did not allow us to establish an explanation for this marked preference, we could suppose that as proteins have no fermentative effect it were consumed more than carbohydrates, such carbohydrates fermentative is associated with fermentative diarrhoea.

Table III
Foods intake frequency evaluated in 42 patients with Crohn's disease

Most daily consumed food	*Times*	*%*	*Most weekly consumed food*	*Times*	*%*	*Most monthly consumed food*	*Times*	*%*
Red meat	2x	30	Soups	2x	20	Fried snacks	1x	35
Milk	2x	25	Pasta with sauce	1x	40	Roasted salted	1x	40
Beans	2x	30	Baked polenta	1x	15	Macaroni with meat	1x	35
Rice	2x	55	Pork	1x	30	Pizza, pancakes...	2x	40
Salt	1x	35	Dried meat	1x	20	Sausage	2x	30
Apple	1x	10	Cold meat	2x	30	Hamburger	1x	35
Papaya	1x	10	Chicken	2x	30	Fish	2x	45
Coffee/tea w sugar	7x	15	Yogurt	1x	35	Lentil	2x	25
Coffee/tea w/o sugar	1x	35	Mozzarella	4x	20	Beans	1x	40
Bread	1x	20	Fresh Cheese	2x	15	Baked potato	3x	20
Milked chocolate	1x	25	Egg	2x	30	Mayonnaise salad	2x	25
			Chip potato	2x	25	Diet soda	1x	5
			Manioc flour	2x	20	Beer	1x	20
			Lettuce	3x	35	Sandwich	1x	20
			Carrot	1x	20			
			Other vegetables	1x	20			
			Cooked vegetables	2x	20			
			Broccoli	2x	25			
			Oils	4x	35			
			Mayonnaise	1x	25			
			Orange	2x	25			
			Banana	3x	20			
			Natural juice	3x	35			
			Industrialized juice	3x	30			
			Regular soda	3x	35			
			Biscuit	3x	20			
			Filled cookie	1x	30			
			Cake	1x	25			
			Bread with butter	3x	25			
			Chocolate	1x	25			
			Dessert	4x	25			
			Sugar	6x	10			

The most frequent complications generated by nutritional deficiencies in inflammatory bowel diseases are anemia and osteoporosis. Osteoporosis is caused by low intakes of calcium and vitamin D; anemia, may be associated with chronic loss, long lasting inflammation and nutritional deficiencies, it is linked to folate and B_{12} vitamin deficiencies.[21]

In clinical practice, assessment of the nutritional status in CD patients has showed a reduction in overall food intake caused by anorexia, nausea, vomiting, abdominal pain, diarrhea and restricted diets, such fact results in nutrients deficiency.[16]

Supplemented patients presented significant increment in lean body mass during 16-week supplementation period, while compared to non supplemented group; such nutritional benefit may be associated with whey protein, since that compared to other protein sources whey protein has a unique ability to increase glutathione production, which results in body composition improvement.[23,24] Several studies have shown beneficial effects of whey protein on body composition in direct comparison with other sources of high quality proteins. Muscle increase and/or preservation not only improves body composition, but also significantly contributes to increase the chances of living a longer and healthier life.[25,26]

Whey proteins are rich in calcium, approximately 600 mg/100 g, and several epidemiological studies have found an inverse relation between calcium intake, from milk and its derivatives, and body fat.[27] A reasonable explanation to this fact is that increased dietary calcium reduces the concentrations of calcitropic hormones,

Table IV
Mean values of BMI, % fat, total fat (kg) and lean body mass (kg), comparing differences in a 16-week period, as found in patients with CD supplemented (S) or not supplemented (NS) with whey protein and TGF-β

Body composition	*Supplemented (S)**			*Non supplemented (NS)***	
	T0	*T1*	*T2*	*T0*	*T2*
BMI	23.1	22.6	22.1	25.1	25.7
Lean Body Mass (kg)	50.43	53.71	55.93	51.4	51.53
% Fat	19.6	17.9	16.6	29.8	30.08
Absolute fat (kg)	13.2	11.9	11	22.5	2.78

*Supplemented:T0 *versus* T1 *versus* T2, *p < 0.05, Friebman test.
**Non supplemented: T0 *versus* T2 p > 0.05, Friebman test.
S-T0 = immediately before anti-TNF-α infusion; T1 = 8 weeks after T0; T2 = 8 weeks after T1.
NS-T0 = immediately before anti-TNF-α infusion; T2 = 16 weeks after T0.
BMI = Body mass index.

especially 1.25 hydroxycholecalciferol. In high concentrations, this hormone stimulates transfer of calcium to adipocytes. In adipocytes, high concentration of calcium lead to lipogenesis while reducing lipolysis levels. Therefore, the suppression of calcitropic hormones mediated by dietary calcium may help decrease fat deposition in adipose tissues.[28] Supplemented patients had an increase in lean mass and decrease in fat while the non supplemented ones showed no change in body composition. The amino acid profile of whey proteins may possibly favor muscle anabolism. Amino acid profile of whey proteins is very similar to the proteins of skeletal muscle, providing almost all amino acids in proportions similar to those needed in skeletal muscle, classifying it as an effective anabolic supplement.[29]

Studies show that whey proteins are absorbed more quickly than others, such as casein, reaching amino acids as leucine to high plasmatic values, immediately after intake.[30] In addition to increasing plasma concentrations of such amino acids, ingestion whey proteins solutions significantly increases the concentration of plasma insulin, favoring amino acids uptake into the muscle cells, optimizing the synthesis and reducing the protein catabolism.[31]

Conclusion

Supplementation with whey protein, was associated to lean body mass increment and body fat reduction. Based on the obtained results, these patients should be advised regarding nutrients intake, focusing on the importance of supplementation

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