Hospitalaria

Original/*Alimento funcional*

Whey and soy protein supplements changes body composition in patients with Crohn's disease undergoing azathioprine and anti-TNF-alpha therapy

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Abstract

Background: **Crohn's disease (CD) is a chronic transmural inflammation of the gastrointestinal tract of unknown cause. Malnutrition associated with active CD has been reduced although obesity has increased. Dietary strategies such as those with high-protein have been proposed to reduce body fat. This study compares the effects of two supplements on the nutritional status of CD patients.**

Materials and Methods: **68 CD patients were randomized in two groups: whey protein group (WP) and soy protein group (SP). Using bioimpedance analysis, anthropometry and albumin and pre-albumin dosages the nutritional status was measured before starting the intervention and after 8 and 16 weeks. The disease activity was determined by Crohn's Disease Activity Index and serum C-reactive protein dosage and dietary intake by 24h dietary recalls.**

Results: **Forty-one patients concluded the study and both supplements changed body composition similarly. Triceps skin fold thickness (p< 0.001) and body fat percentage (p=0.001) decreased, whereas mid-arm muscle circumference (p=0.004), corrected arm muscle area (p=0.005) and body lean percentage (p=0.001) increased.**

Conclusions: **For Crohn's disease patients undergoing anti TNF-alpha and azatioprine therapies, supplementation with whey and soy proteins changes body composition through reduction of body fat and thus contributes to control inflammation.**

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Key words: *Crohn's Disease. Whey Protein. Soy Protein. Body Composition.*

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SUPLEMENTOS DE PROTEÍNA DE SUERO Y SOYA CAMBIA LA COMPOSICIÓN CORPORAL EN LOS PACIENTES CON ENFERMEDAD DE CROHN SOMETIDOS CON AZATIOPRINA Y ANTI-TNF-ALFA TERAPIAS

Resumen

Introducción: **La enfermedad de Crohn (EC) es un trastorno inflamatorio crónico transmural del tracto gastrointestinal de carácter desconocida. La desnutrición asociada con EC activa se ha reducido a pesar de la obesidad que ha aumentado. Se han propuesto estrategias dietéticas, como aquellos con alto contenido de proteínas para reducir la grasa corporal. Este estudio compara los efectos de dos suplementos sobre el estado nutricional de los pacientes con EC.**

Materiales y Métodos: **Fueron randomizados en dos grupos 68 pacientes con EC: el grupo de proteína de suero y el grupo de proteína de soya. Se utilizo el análisis de bioimpedancia eléctrica, la antropometría y dosificaciones de albúmina y prealbúmina del estado nutricional midiéndose antes de comenzar la intervención y después de 8 y 16 semanas. La actividad de la enfermedad se determinó por Índice de Actividad de Enfermedad de Crohn (CDAI), dosificación en suero de la proteína C reactiva y la ingesta dietética por recordatorio de 24h.**

Resultados: **Cuarenta y un pacientes concluyeron el estudio y ambos suplementos cambiaron la composición corporal de manera similar. El espesor del pliegue cutáneo del tríceps (p <0,001) y el porcentaje de grasa corporal (p = 0,001) se redujeron, mientras que la circunferencia muscular braquial (p = 0,004), el área muscular del brazo corregida (p = 0,005) y el porcentaje corporal magra (p = 0,001) han aumentado.**

Conclusiones: **En los pacientes con enfermedad de Crohn sometidos con anti-TNF-alfa y terapias azatioprina, la suplementación con proteínas de suero de leche y de soya cambia la composición corporal a través de la reducción de la grasa corporal y por lo tanto contribuye para controlar la inflamación.**

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Palabras clave: *Enfermedad de Crohn. Proteína de suero. Proteína de soja. La composición corporal.*

Background

Crohn's disease (CD) is a chronic inflammatory disease of the gastrointestinal tract. Complications such as obstruction, fistulas and abscesses can appear during the course of the disease[1,2].

The CD Activity Index (CDAI) and the Harvey-Bradshaw Index (HBI) have been recommended to assess CD activity. The CDAI has been considered the gold standard for clinical trials[3]. However, this index does not take into account objective measures of inflammation such as C-reactive protein (CRP) and endoscopic lesions. A recent study showed that the CDAI is not a reliable measure to predict endoscopic and/or CRP remission among patients under azathioprine and/or infliximab[4]. This result indicates that the index will probably be updated or changed.

CD is traditionally associated with weight loss, but with the development of new therapies and early interventions this may be changing. Nowadays, obesity has also occurred[5,6]. The mechanisms underlying the putative effects of overweight and obesity on CD are unknown. It has been demonstrated that a production of proinflammatory cytokines from adipose tissue such as tumor necrosis factor alpha (TNF-alpha), interleukin six (IL-6) and adipokines are elevated in obesity[7]. This inflammatory state may have detrimental effects on CD, creating a favorable environment for gut inflammation and progression of disease[8,9,10].

Intake of protein has been shown to influence satiety, thermogenesis and body composition. Soy protein (SP) reduced body fat in obese rats and whey protein (WP) in obese humans has the same effect[11-14]. The study with WP intake in CD patients was the open-label which showed reduction in body fat[15]. WP intake has stimulated glutathione synthesis in HIV infected children[16], reduced IL-6 and increased glutathione in patients with ischemic stroke[17].

Based on the results of our previous open-label study[15], the aim of the present study was to compare the effect of whey Protein (WP) and soy Protein (SP) supplements on body composition and disease activity in CD patients.

Methods

Trial design

This was a randomized, parallel-arm, 16-week study, conducted in line with CONSORT recommendations. Patients with Crohn's Disease were recruited from February to October 2012 in the Inflammatory Bowel Disease outpatient clinic at the University Medical School of Campinas, SP - Brazil. The sample size was based on the results of our previous open-label[15] and calculated to determine a 15% change in body fat percentage (P=0.05) with 80% power among each group comparison.

Patients

The trial included CD patients treated with azathioprine or anti-tumor necrosis factor-alpha (anti-TNF-alpha) or azathioprine and anti-TNF-alpha and excluded smokers and those who were using other medication or nutritional supplements.

Trial protocol

The protocol of the study was approved by the Ethics Committee of the University of Campinas (CAAE: 10990146000-11) and registered on ClinicalTrials.gov. Identifier: NCT01957423.

A random number sequence, generated by Microsoft Office Excel 12.0 (Office 2007), was used to designate the treatment assignment. We used blocks of size 2 at random to create the allocation sequence. Patients were randomized 1:1 for each group: one supplemented with a concentrate of whey protein (WP) donated by Hilmar Cheese and the other, with an isolate soy protein (SP) from Probiótica (compositions specified in table I). Randomization and allocation were conducted by the "blinded" researcher.

WP and SP were provided as identical sachets with 15g and 12g of supplement, respectively. Sachets were packed in bags with a code for each supplement, A or B. Both patients and researchers were unaware of the treatment assignation. Patients were advised to consume two sachets per day (amounting to 22.4g of protein) mixed with food or a cold beverage immediately after opening the sachet, for 16 weeks. All patients remained on an unrestricted diet and did not receive nutritional advice.

Compliance was checked every two weeks by phone and also by counting the number of packets not consumed.

Follow-up Assessment

The body composition in the three evaluations was measured at the same period of the day, using bioimpedance analysis. Whole-body resistance and reactance were measured using a tetrapolar bioelectrical impedance analyzer in accordance with the manufacturer's guidelines (BIODYNAMICS, Model 310e). This analyzer provides data on lean mass and fat mass. Nutritional status was additionally assessed using anthropometric parameters. Body weight was measured by a medical decimal scale to an accuracy of 0.01 kg. Body height was measured to the nearest millimeter using a stadiometer. Body mass index (BMI) was calculated from weight and height (kilograms per meter squared) and classified according to the World Health Organization. Mid-arm circumference (MAC) and triceps skin fold thickness (TSF) were obtained using the tape measure and Lange skin fold caliper. Mid-arm

Table I
Chemical composition of the whey protein (WP) and soy protein (SP) treatment supplements[1]

	WP	*SP*
	g/100g	
Protein	73.5	90.0
Moisture	6.3	6.0
Total fat	13.9	0.5
Total carbohydrate	6.6	1.0
Alanine	5.0	3.9
Arginine	3.3	7.3
Cystine	2.3	1.3
Phenylalanine	3.5	5.1
Glycine	2.1	3.9
Histidine	1.9	2.2
Isoleucine	5.8	4.2
Leucine	10.3	7.6
Lysine	8.6	5.9
Methionine	3.1	1.2
Proline	5.9	5.5
Serline	5.5	5.0
Tyrosine	3.2	3.5
Threonine	6.3	3.4
Tryptophan	1.1	1.5
Valine	5.3	4.6
Asparticacid	10.9	11.2
Glutamicacid	16.9	20.5
Isoflavone	0.0	0.2

[1]Chemical compositions were determined by Institute of Food Technology, São Paulo, Brazil and Department of Food and Nutrition, Faculty of Food Engineering, State University of Campinas, São Paulo, Brazil.

muscle circumference (MAMC) and corrected arm muscle area (CAMA) were calculated from MAC and TSF. All measurements were obtained by the same observer according to the National Health and Nutrition Examination Survey and results were compared with reference values provided by Frisancho AR[18].

For the evaluation of disease activity, the Crohn's Disease Activity Index – CDAI[19] was used. Blood samples were drawn to determine biochemical parameters, serum albumin, pre-albumin, C-reactive protein (CRP) and hemogram, measured by routine methods in use at the University of Campinas Medical School.

The dietary intake was assessed by 24h recalls, before the intervention and after 8 and 16 weeks. Food portion sizes were estimated by the patients with the help of photos shown to them. Macronutrients and micronutrients were calculated with the help of software for nutrient evaluation (Dietpro 5.i). Tables of the Dietary Reference Intakes – DRIs were used to evaluate the adequacy of the nutrient intake.

Statistics

Statistical analyses were performed using SPSS 16.0 software package. The correlations between the biochemical and clinical measurements were calculated employing a bivariate correlation with a non-parametric distribution (Spearman's rho). Comparison of categorical data between the groups was performed using the χ^2 method or Fisher exact test. Comparison of continuous data between the groups was performed using the Student t test for the normal data or Mann-Whitney test for the non-normal data. One-Way Repeated ANOVA was used for comparison of continuous data between the different times in which the patients were evaluated. For non-normal data the Friedman test was used. A level of 5% significance was established.

Results

Patient cohort

The flowchart of the randomized trial is shown in figure 1. Forty-one patients (19 of WP, 22 of SP) completed the 16-week supplementation period. Clinical, nutritional and demographic data at baseline of these patients are in table II. There were no statistical differences between the groups, table III.

Biochemical and clinical outcomes

There was no significant difference between groups (before, during and after the nutritional supplementation) to albumin, pre-albumin, CRP and CDAI. There was a significant positive correlation between CDAI and CRP ($r = 0.36$, $P = 0.019$) of the two groups. Furthermore, CDAI and CRP were negatively correlated with albumin ($r = -0.39$, $P = 0.012$; $r = -0.54$, $P < 0.001$) and pre-albumin ($r = -0.31$, $P = 0.046$; $r = -0.65$, $P < 0.001$).

Nutritional outcome

BMI was similar for both groups. At the first evaluation, 19.5% of patients were overweight and 9.8% obese and only one was malnourished.

The two supplements changed body composition. Body fat percentage (Fig. 2A) and TSF (Fig. 2C) decreased, whereas body lean percentage (Fig. 2B), MAMC (Fig. 2D) and CAMA (Fig. 2E) increased.

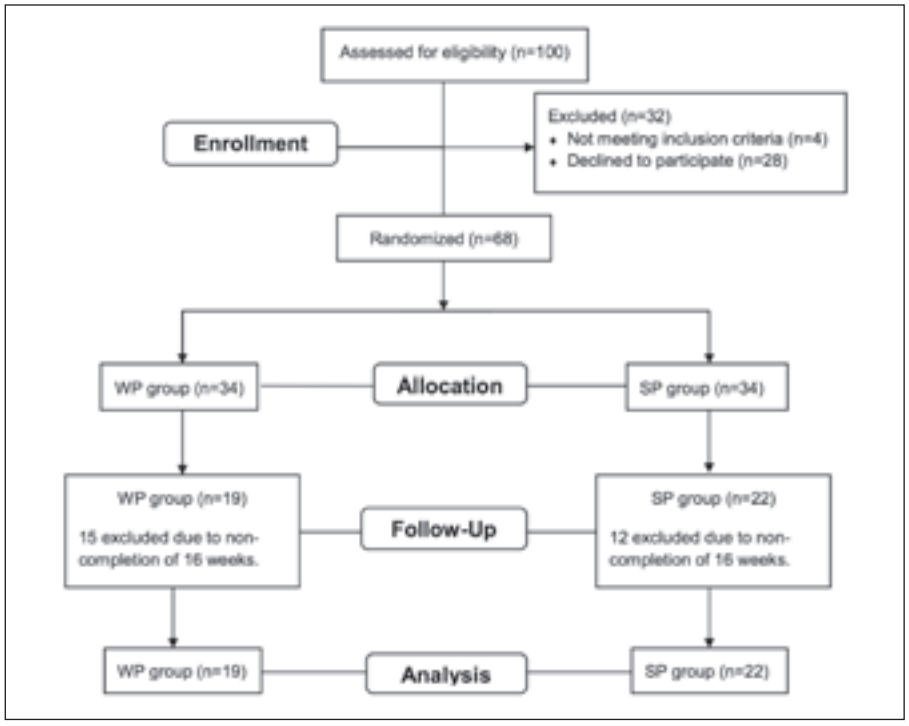

Fig. 1.—Flow chart of the randomization of the two groups.

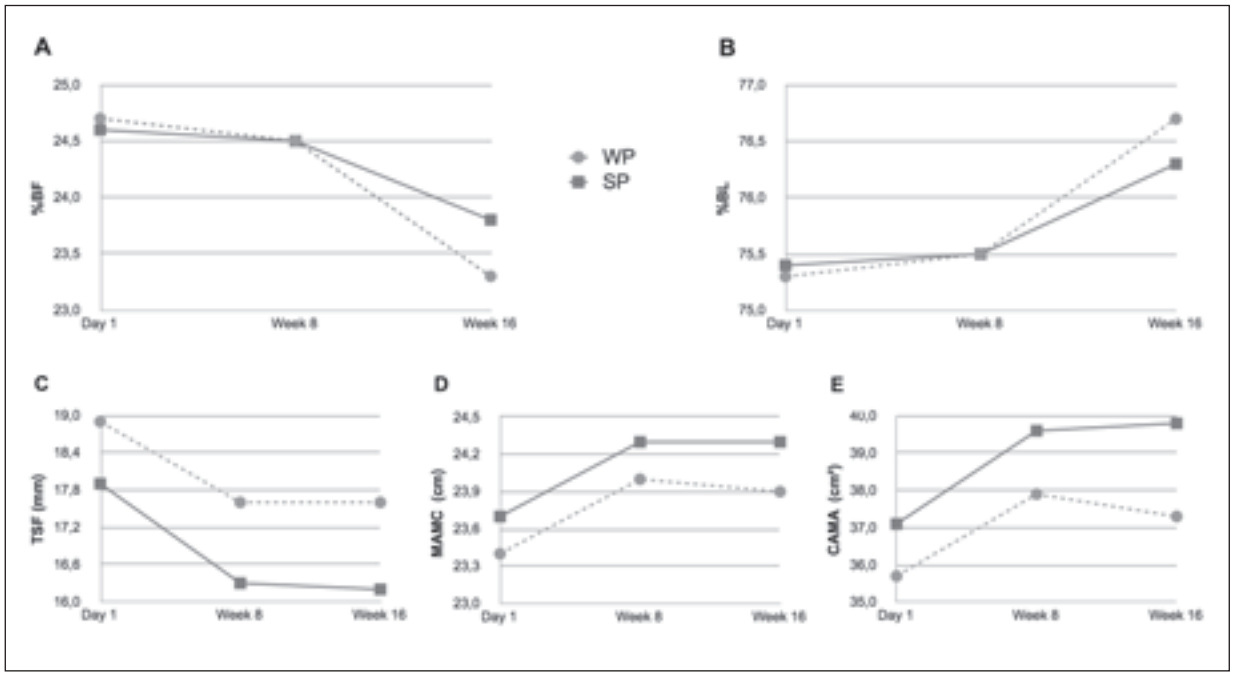

Fig. 2.—Effect of whey protein and soy protein. A-body fat percentage (%BF); B-body lean percentage (%BL); C-triceps skin fold (TSF); D-mid-arm muscle circumference (MAMC); E-corrected arm muscle area (CAMA). There were significant differences within groups (ANOVA).

Dietary Intake

Intake of micronutrients did not change during the study. Considering the two groups, the daily energy of the first dietary recall was 1962.8 ± 646.0 kcal/day and the percentage of carbohydrate, protein and lipids relative to total energy were, respectively: 49.1± 8.4%; 18.3 ± 5.1%; 32.6 ± 7.6%, which were in accordance with the DRIs.

We observed some deficiencies of the micronutrient intake in comparison with the DRIs. Figure 3 shows the proportion of patients whose nutrient intake was below the recommendation in the first evaluation.

Patients who withdrew from the study

Twenty-seven patients did not complete the study (10 men and 17 women). Clinical, nutritional and demographic data at baseline of these patients are in table II. Of these patients, 51.9% were under anti-TNF-alpha therapy, 33.3% were under azathioprine therapy and the remainder used both drugs. Concerning BMI, 51.9% were eutrophic, 33.3% overweight, 7.4% obese and 7.4% undernourished. Only one patient had a CDAI greater than 150 (166), and BMI of 36.5 kg / m^2. They had a higher mean TSF (p = 0.02) than the group that completed the study (Table II). After 16 weeks the excluded group did not show changes in CRP (0.6 ± 0.9mg/dL, p=0.5) or CDAI (64.21 ± 68.8, p=0.9) mean. The reasons why these patients left the study were: 11 did not like the taste, 8 had diarrhea and nausea, 6 did not follow the intake schedule and 2 had skin reaction.

Discussion

The results of this randomized study demonstrated that nutritional supplementation with WP or SP in patients with CD changed body composition but had no

Table II
Characteristics of the patients at baseline

Characteristics	*Patients who completed the study (n=41)*	*Patients who completed the study (n=27)*	*p Value*[1]
Mean (SD) age, years	40.2 (10.8)	38.3 (12.8)	0.51
Male, n (%)	29 (70.7)	10 (37.0)	0.06
Mean (SDD) duration of CD[2] years	10.3 (6.8)	11.4 (7.1)	0.53
Mean (SD) CDAI[3]	73.8 (69.7)	56.0 (43.1)	0.11
Resection[4], n (%)	21 (51.2)	18 (66.7)	0.21
Disease site			
Colon/rectum/anal canal, n (%)	14 (34.1)	6 (22.2)	
SB[5], n (%)	15 (36.6)	13 (48.1)	0.39
Colon/rectum/anal canal, + SB[5], n (%)	12 (29.3)	8 (29.6)	
Medication			
Azathioprine, n (%)	22 (53.7)	9 (33.3)	
Anti-TNF-α, n (%)	14 (34.1)	14 (51.9)	0.25
Azathioprine + Anti-TNF-α, n (%)	5 (12.2)	4 (14.8)	
Laboratory parameters			
Mean (SD) CRP[6], mg/dL	0.8 (1.0)	0.6 (0.9)	0.30
Mean (SD) Albumin, g/dL	4.3 (0.6)	4.4 (0.4)	0.66
Mean (SD) Pre-albumin, mg/dL	30.3 (9.0)	28.8 (10.1)	0.52
Mean (SD) Homoglobin, g/dL	13.2 (1.8)	13.5 (1.5)	0.50
Mean (SD) Hematocrit, %	39.3 (6.1)	41.1 (4.0)	0.18
Nutritional parameters			
Mean (SD) BMI[7], kg/m^2	24.1 (3.5)	24.1 (4.9)	0.98
Mean (SD) MAC[8], cm	29.3 (3.3)	29.7 (3.8)	0.65
Mean (SD) TSF[9], mm	18.4 (6.5)	23.2 (10.0)	0.02*
Mean (SD) MAMC[10], cm	23.5 (3.7)	22.4 (3.2)	0.19
Mean (SD) CAMA[11], cm^2	36.4 (13.9)	33.1 (10.0)	0.29
Mean (SD) %BF[12]	24.7 (6.8)	28.0 (8.5)	0.08

[1]p Values calculated by the Student T test for continuous data and X^2 test or Fisher exact test for categorical data. [2]CD, Crohn's disease; [3]CDAI, Crohn's Disease Activity Index; [4]number of patients underwent bowel resection; [5]SB, small bowel; [6]CRP, C- reactive protein; [7]BMI, Body Mass Index; [8]MAC, Mid-arm circumference; [9]TSF, triceps skin fold; [10]MAMC, mid-arm muscle circumference; [11]CAMA, corrected arm muscle area; [12]%BF, body fat percentage.

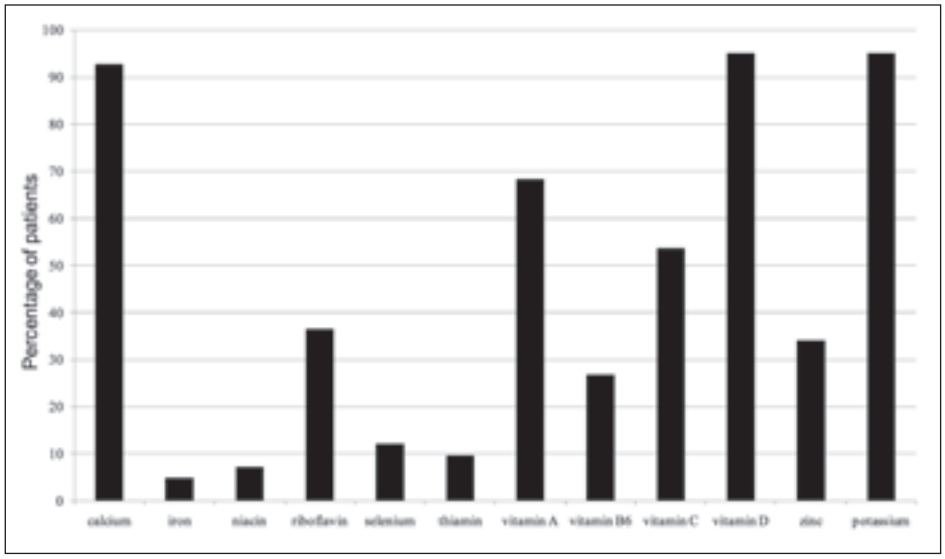

Fig. 3.—Proportion of CD patients with nutrient intakes below DRIs.

Table III
Comparsion of WP and SP groups that completed the study, before intervention

Characteristics	*WP (n=19)*	*SP (n=22)*	*p Value*[1]
Mean (SD) age, years	40.8 (12,4)	39.6 (9.4)	0.74
Male, n (%)	15 (78.9)	14 (63.6)	0.28
Mean (SDD) duration of CD[2] years	9.3 (6.2)	11.1 (7.2)	0.40
Mean (SD) CDAI[3]	86.4 (73.6)	62.9 (65.8)	0.07
Mean (SD) CRP[4], mg/dL	0.75 (0.8)	0.76 (1.2)	0.69
Resection[5], n (%)	9 (47.4)	12 (54.5)	0.65
Disease site			
Colon/rectum/anal canal, n (%)	9 (47.4)	5 (22.7)	
SB[6], n (%)	5 (26.3)	10 (45.5)	0.23
Colon/rectum/anal canal + SB[6], n (%)	5 (26.3)	7 (31.8)	
Medication			
Azathioprine, n (%)	11 (57.9)	11 (50)	
Anti-TNF-α, n (%)	6 (31.6)	8 (36.4)	0.88
Azathioprine + Anti-TNF-α, n (%)	2 (10.5)	3 (13.6)	

[1]p Values calculated by the Student t test for continuous data and X^2 test or Fisher exact test for categorical data. [2]CD, Crohn's disease; [3]CDAI, Crohn's Disease Activity Index; [4]CRP, C-reactive protein, [5]number of patients underwent bowel resection; [6]SB, small bowel

effect on the disease activity. Several studies have evaluated the efficacy of enteral formulations to control the disease activity in CD patients[20,21]. In our study, most patients were in remission and the CDAI remained unchanged throughout the study. We observed a weak but significant correlation of CRP with CDAI. Other authors demonstrated an association of CRP with clinical activity as well as endoscopic and histologic inflammation[22,23]. Therefore, both parameters can be used in clinical practice to assess the activity of Crohn's disease.

In 2008 and 2009 data were collected from 188,000 Brazilian people of which 50% of men and 48% of women were overweight, and 12.5% of men and 16.9% of women were obese[24]. We found 19.5% overweight and 9.8% obese in Brazilian CD patients who tend to have the same lifestyle and dietary habits of the general population.

Recent research has shown an increasing prevalence of overweight and obesity in IBD patients[5,6]. Steed et al.[6] observed 18% obese and 38% overweight in 489 patients. Obesity and CD were associated with development of mesenteric fat, which produces proinflammatory adipokines[25]. The excess of these adipokines in CD patients with obesity may have adverse effects during the course of the disease[9].

Our analysis of the food consumed revealed that the energy and macronutrient intakes were adequate, but many patients had inadequate consumption of fiber, calcium, potassium and vitamins A, C and D. This has also been observed in other studies[26,27]. The reason is that many of these patients have lactose intolerance and avoid the consumption of milk and other dairy products. Furthermore, diets that exclude vegetables and fruit are often recommended to improve gastrointestinal symptoms in patients with active disease and the majority of patients continue with this diet even after remission[26,28]. Oral supplements of calcium and vitamin D are often required due to the risk of bone disease.[28] Diet counseling is important and has been effective in the repairing of nutrient deficiencies[29].

WP mixture supplementation for 12 weeks in obese subjects without CD led to higher reductions of body fat than the control group who consumed glucose[12]. Baer et al.[13] compared the supplementation of obese individuals with WP, SP and carbohydrates. At the end of the study the WP group had less body fat than the carbohydrate and SP groups. Our study did not show differences in the body composition between the WP and SP groups. However, the two protein sources produced the same effects in body composition after 16 weeks of supplementation, a reduction in body fat and an increase in lean mass.

Although the absence of a placebo group leads to limitations in the study, no placebo was found with similar organoleptic characteristics of supplements. Another drawback was that there were 17 drop-out patients although clinical and laboratory differences between them and those who completed the study were not detected. Moreover, even after leaving the study, the patients did not change their clinical status. Previously, the acceptability of this WP supplement was evaluated[30], and it was concluded that adherence to supplementation could be increased with an improvement in its organoleptic characteristics.

Conclusion

For Crohn's disease patients, supplementation with whey and soy proteins as an additional therapeutical intervention changes body composition through reduction of body fat and thus contributes to control inflammation.

Competing interests The author(s) declare that they have no competing interests.

Authors' contributions

JFM, CSRC, AMM and MMSV participated in the design of the study and performed the statistical analysis; JFM, VO and SDS collected data; CW provided essential material (Whey Protein); JFM and MMSV drafted the manuscript. All authors read and approved the final version of the manuscript.

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