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Original Article

A double-blind, randomized and placebo-controlled clinical trial with *Agaricus sylvaticus* fungus in anthropometric profile of women with colon cancer

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ABSTRACT

Introduction: Colorectal cancer is a disease influenced by genetic and environmental factors. Medicinal fungi and/or its extracts have been used in the adjuvant therapy of cancer because of their pharmacological, nutritional and immunomodulatory properties.
Objective: To evaluate the anthropometric profile of colorectal cancer women after dietary supplementation with *Agaricus sylvaticus* fungus.
Methods: Randomized, double-blind, placebo-controlled clinical trial was conducted in a public hospital in the Federal District – Brazil for six months. Sample of 32 patients with colorectal cancer, female, was separated into two groups: supplemented with *Agaricus sylvaticus* (30 mg/kg/day) and placebo. We conducted anthropometry (weight, height, body mass index, arm circumference, triceps skinfold, arm muscle circumference and fat percentage) during the treatment. The results were analyzed at three different times (before the start of treatment, three months and after six months supplementation) using the Microsoft Excel 2007 and SPSS 19.0, using Student's *t*-test and *F*, with significance for $p \leq 0.05$.
Results: The *Agaricus sylvaticus* group showed a significant increase in body mass index, arm circumference, percent body fat and triceps skinfold, and non-significant increase in arm muscle circumference after six months of supplementation. These results were not observed in the placebo group.
Conclusion: The results suggest that dietary supplementation with *Agaricus sylvaticus* is capable to have benefits in anthropometric parameters of women with colorectal cancer.
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Ensaio clínico duplo cego, randomizado e placebo controlado com fungos *Agaricus sylvaticus* no perfil antropométrico de mulheres com câncer colorretal

Palavras-chave:
Antropometria
Câncer colorretal
Fungos *Agaricus sylvaticus*

RESUMO

Introdução: O câncer colorretal é uma doença influenciada por fatores genéticos e ambientais. A utilização de fungos medicinais e/ou de seus extratos tem sido utilizada no adjuvante tratamento do câncer devido às suas propriedades farmacológicas, nutricionais e imunomoduladoras.
Objetivo: Avaliar o perfil antropométrico de mulheres com câncer colorretal após suplementação dietética com fungos Agaricus sylvaticus.
Métodos: Ensaio clínico randomizado, duplo-cego, placebo-controlado realizado em um hospital público do Distrito Federal Brasil por seis meses. Amostra constituída por 32 pacientes com câncer colorretal, sexo feminino, separados em dois grupos: suplementado com Agaricus sylvaticus (30 mg/kg/dia) e placebo. Realizou-se a antropometria (peso, estatura, índice de massa corporal, circunferência do braço, dobra cutânea tricipital, circunferência muscular do braço e percentual de gordura) ao longo do tratamento. Os resultados foram analisados em três momentos distintos (antes do início do tratamento, com três meses e após seis meses de suplementação), utilizando os programas Microsoft Excel 2007 e SPSS 19.0, por meio dos testes T-student e F, com significância para $p \leq 0{,}05$.
Resultados: O grupo Agaricus sylvaticus apresentou aumento significativo de índice de massa corporal, circunferência do braço, percentual de gordura corporal e dobra cutânea triciptal e, aumento não significativo de circunferência muscular do braço após seis meses de suplementação. Esses resultados não foram observados no grupo placebo.
Conclusão: Os resultados sugerem que a suplementação dietética com Agaricus sylvaticus é capaz de exercer benefícios nos parâmetros antropométricos de mulheres com câncer colorretal.

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Introduction

Nowadays, due to its increasing incidence, cancer has become a public health problem worldwide,[1] *pari passu* with the progressive aging of the population, as a consequence of an increased life expectancy.[2]

Colorectal cancer is a common and deadly disease, influenced by genetic and environmental factors and also by the mutual influence of both. Genetic predisposition is a predominant risk factor for some individuals; however, environmental factors, including diet, physical activity, smoking and obesity, are also included among high-risk factors.[2]

As for the risk of developing colorectal cancer, patients can be divided as follows: those less than 50 years and no family history of colorectal cancer are at low risk; those aged 50 or more and with no other risk factors are included in the average risk group; patients with personal history of polyps or colorectal cancer, or with a family history of colorectal cancer or with first-degree relatives diagnosed with polyps are classified as high-risk people; and finally, the very high-risk classification comprises those patients with polypoid syndromes, or who are suffering from inflammatory bowel disease.[3]

Most often, a diagnosis of cancer leads to a phase of much anxiety and distress, possibly triggering a picture of depression. In turn, the depression comes in association with somatic symptoms such as loss of appetite and fatigue, which may also be associated with the catabolism and/or treatment of the disease.[4]

The use of medicinal fungi and/or their extracts as dietary supplements has increased considerably, thanks to its anti-tumor, anticarcinogenic, antiviral, anti-inflammatory, hypoglycemic, hypocholesterolemic and hypotensive effects, among others, and these products may be recommended as adjuvants in the treatment of malignant neoplasms.[5]

Considering the prominence of this theme, this study aimed to evaluate the anthropometric profile of women with colorectal cancer after dietary supplementation with the fungus *Agaricus sylvaticus*.

Methods

Study design

The study consists of a randomized, double-blind, placebo-controlled study, which was approved by the Ethics Committee on Human Research, State Secretariat of Health, Distrito Federal (CEP/SES/DF) under Protocol 051/04. The patients' free and informed consent (FIC) was obtained, and their participation was voluntary. The study was conducted at the Proctology Outpatient Clinic, Hospital de Base do Distrito Federal, Brazil, between November 2004 and July 2006.

The randomization procedure occurred through sequential numbers randomly generated by computer, where each random number corresponded to a group receiving the fungus (Group A) or placebo (Group B). These numbers were inserted into opaque, not translucent and closed envelopes, with the generation of the number sequence performed by a researcher blinded to the study, after selection of patients with inclusion and exclusion criteria. The envelopes were opened sequentially as the patients were consecutively recruited for the study and contained the group to which the patient would belong. Only after performing the statistical analysis, it was revealed which group had received placebo and which received *Agaricus sylvaticus*.

Patients

The sample consisted of patients with colorectal cancer divided into two groups: those who received placebo and those supplemented with *Agaricus sylvaticus*. The following inclusion criteria should be fulfilled: female patients with a confirmed diagnosis of colorectal cancer in the postoperative phase, from three months to two years of surgery, and older than 20 years. Exclusion criteria were: pregnant women, breastfeeding mothers, bedridden individuals, physically disabled people, patients using an alternative therapy or with other chronic non-communicable diseases, and in metastasis process.

Agaricus sylvaticus extract

With a widespread geographical distribution and naturally occurring in Brazil, *Agaricus sylvaticus* was first described in Switzerland. Its identification was confirmed by the London Royal Botanic Gardens, whose documentation was provided by the Instituto de Botânica, Environment State Secretariat, São Paulo, in November 10, 1995. The *Agaricus sylvaticus* fungus (Family: *Agaricaceae*), whose popular name is Sun Mushroom, was obtained from a producer duly accredited by the Empresa Brasileira de Pesquisa Agropecuária – Embrapa, from Tapiraí, State of Sao Paulo, Brazil. The fungus extract was obtained by soaking the dehydrated material in hot water during 30 min; then, the material was liquified, sieved and dried in a desiccator. The analysis of *Agaricus sylvaticus* composition was performed by the Japan Food Research Laboratories Center and revealed the presence of carbohydrates (18.51 g/100 g), lipids (0.04 g/100 g), ergosterol (624 mg/100 g), proteins (4.99 g/100 g), amino acids (arginine – 1.14%; lysine – 1.23%, histidine – 0.51%, phenylalanine – 0.92%, tyrosine – 0.67%, leucine – 1 43% methionine – 0.32%, valine – 1.03%, alanine – 1.28% glycine – 0.94%, proline – 0.95%, glutamic acid – 3.93%, serine – 0 96%, threonine – 0.96%, aspartic acid – 1.81%, tryptophan – 0.32% cysteine – 0.25%) and trace amounts of micronutrients.

The dry extract was transformed into tablets, in accordance with pharmacotechnical procedure. The dosage of the fungus administered to patients from the supplemented group was equivalent to 30 mg/kg/day, divided into two daily doses (six tablets a day, three in the morning and three in the afternoon, in between meals), considering the mean weight of the study population over a period of six months. As for the group of patients who received placebo, the tablets were administered in the same quantities, with the same excipients and energy, but without the extract of *Agaricus sylvaticus* (in its place, the placebo group received starch).

Clinical evolution

Patients were followed for six months. During the first three months, the visits were held fortnightly for clinical assessment and, in the last three months, the visits were held every 30 days.

The food anamnesis (semiquantitative and 24-h recall food consumption frequency questionnaire) was held on the first and last days of consultation. However, the patients were instructed to remain with the usual diet, in order not to interfere with the intervention, although during treatment they have received guidelines on how to maintain a healthy diet. After a 6-month follow-up, an individualized diet was suggested for all patients, who, when necessary, were referred to other health professionals.

The anthropometric assessment was performed using body mass index (BMI), triceps skinfold thickness (TSF), arm circumference (AC), arm muscle circumference (AMC) and body fat percent (%BF). However, for statistical purposes, we extracted the average of the results obtained in three different times: before starting supplementation, after three months of treatment and after six months of treatment.

All patients were followed weekly by researchers to clarify any doubts, check on the proper use of the mushroom and for confirmation of the schedule, ensuring greater adherence to treatment and control on the continuity of the study.

We considered as dropouts those patients who did not attend the consultations during the full period of six months. Those patients who died before the end of treatment were excluded from the sample.

Anthropometric assessment

A special form of anthropometric assessment, to be filled in all the consultations, was used. Weight determination was performed with the patient barefooted, wearing light clothing and without jewellery interfering with the measurement results. The patient should remain standing in the center of the scale, with her body weight equally distributed between both feet.[6] In order to obtain this variable, a Plenna® – Resolve digital scale (MEA-02500 model) with bioimpedance (BIA), capacity of 150 kg, with 0.1 kg variation and properly calibrated was used.

For height measurement, the barefooted patient should stay upright and in an erect position, with her body lifted at maximum extension, head up, looking forward, in a Frankfurt position, with her back and the back of her knees touching the wall and with feet together.[6] The Frankfurt anatomical plane extends from the bottom margin of the eye socket to the top border of the auditory canal.[7] Patients' height was measured only once, in centimeters (cm), with a 150-cm long inelastic measuring tape attached to a flat wall without baseboard and fixed at 50 cm from the ground. A wood square was placed on top of the head of the patient; with this, we obtained a measure with 0.1-cm accuracy.

After data acquisition (weight and height), BMI was obtained by dividing the patient's weight in kilograms by her

height in meters squared. BMI values <18.5 kg/m^2 would characterize thinness; ≥18, 5 kg/m^2 and <25 kg/m^2, normal weight; ≥25 kg/m^2 and <30 kg/m^2, overweight; and ≥30 kg/m^2, obesity, according to the classification recommended by World Health Organization.[8]

TSF was measured using a Cescorf® quick-reading compass, with a range up to 60 mm and accuracy of ±1 mm. Three consecutive measurements were obtained from TSF, and the arithmetic average of the measured values was considered. As for AC measurement, an inextensible-material, 150-cm length, 1-cm scale measuring tape was used. The AMC value was obtained by the formula: AMC = AC − (0.314 × TSF).[8]

TSF, AC and AMC measures were compared to a Frisancho's[9] reference standard, and the adequacy was calculated by dividing the values obtained by the 50th percentile and multiplying the result by 100. As to the nutritional status classification, the following values were considered: obesity: >120%, overweight: 110–120%, normal weight: 90–110%, mild malnutrition: 80–90%, moderate malnutrition: 70–80% and severe malnutrition: <70%.[10]

The body fat percentage (%BF) was obtained also using the Plenna® digital scale.

Statistical analysis

The presented values were compared and analyzed applying *t*-Student and *F* statistical tests, using Microsoft Excel 2007 and SPSS (Statistical Package for the Social Sciences, SPSS Inc, Chicago, USA) for Windows, version 19.0. The accepted statistical significance probability was $p < 0.05$.

Results

After a follow-up of six months in the Proctology Outpatient Clinic, Hospital de Base do Distrito Federal, a total of 40 women with colorectal cancer who met the inclusion and exclusion criteria agreed to participate in the research, and, of these patients, two died and six dropped out for various reasons. The final sample consisted of 32 patients with a mean age of 56.66 ± 14.07 years, at stages I ($n = 4$), II ($n = 12$) and III ($n = 16$), separated into groups receiving placebo ($n = 16$) and *Agaricus sylvaticus* ($n = 16$). The mean ages were 57.67 ± 13.42 years and 55.87 ± 15.11 years for placebo and *Agaricus sylvaticus* supplemented groups, respectively, with no difference between groups ($p = 0.39$).

As for the body mass index, we observed that the placebo group had an initial BMI of 24.25 ± 5.33 kg/m^2; after three months, a significant increase in BMI was noted (from 24.25 ± 5.33 kg/m^2 to 24.40 ± 5.15 kg/m^2, $p = 0.01$) and in the sixth month, there was a further increase (from 24.25 ± 5.33 kg/m^2 to 24.71 ± 4.73 kg/m^2, $p = 0.06$), but this last result was not statistically significant (Fig. 1).

The supplemented group showed an initial BMI of 24.44 ± 4.59 kg/m^2; after three months, a significant increase to 24.91 ± 4.18 kg/m^2 ($p = 0.02$) was observed, and after six months a further significant increase to 25.16 ± 3.92 kg/m^2 occurred, compared to baseline ($p = 0.02$) (Fig. 1).

Regarding arm circumference (AC), in the placebo group an initial value of 28.68 ± 5.80 cm for this variable was observed; after three months, this value had suffered a significant decline, to 27.88 ± 4.56 cm ($p = 0.05$) and, after six months, a non-significant increase, to 28.39 ± 4.39 cm ($p = 0.31$), was observed (Fig. 2).

The *Agaricus sylvaticus* group presented initial values of 28.66 ± 4.19 cm for AC, with a significant increase over three (29.47 ± 4.10 cm, $p = 0.01$) and six (29.68 ± 3, 74 cm; $p = 0.0001$) months of supplementation (Fig. 2).

As for tricipital skinfold thickness, it was observed that the placebo group had an initial mean of 19.53 ± 8.27 mm for

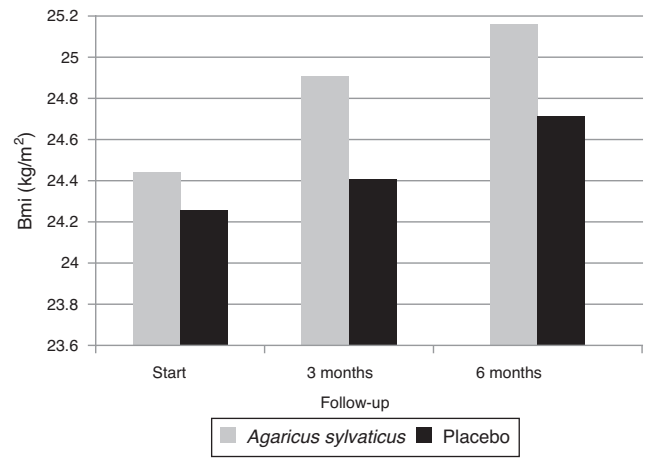

Fig. 1 – Evolution of body mass index (BMI) of women with colorectal cancer in placebo ($n = 16$) and *Agaricus sylvaticus* ($n = 16$) groups, treated at the Proctology Outpatient Clinic, Hospital de Base do Distrito Federal, throughout the clinical follow-up. Placebo: $p = 0.01$ and $p = 0.06$ and *Agaricus sylvaticus*: $p = 0.02$ and $p = 0.02$, after three and six months, respectively. Student's *t*-test.

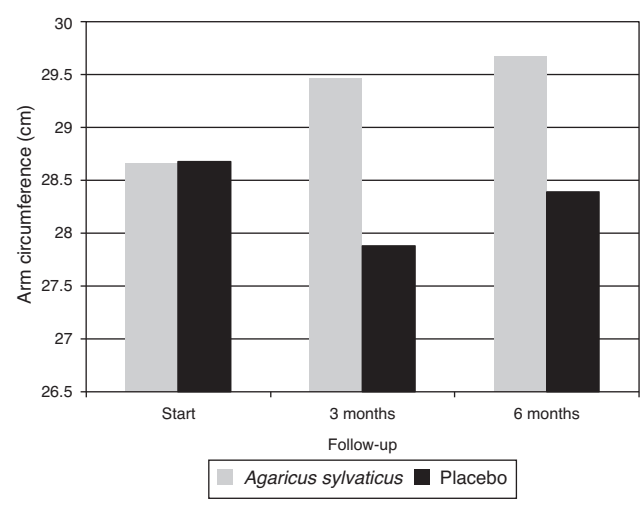

Fig. 2 – Arm circumference (AC) of women with colorectal cancer of placebo ($n = 16$) and *Agaricus sylvaticus* ($n = 16$) groups treated at the Proctology Outpatient Clinic, Hospital de Base do Distrito Federal, throughout the clinical follow-up. Placebo: $p = 0.05$ and $p = 0.31$ and *Agaricus sylvaticus*: $p = 0.01$ and $p = 0.0001$, after three and six months, respectively. Student's *t*-test.

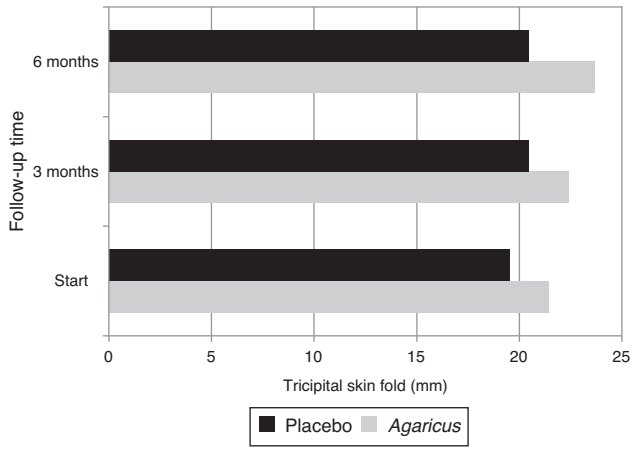

Fig. 3 – Tricipital skin fold (TSF) of women with colorectal cancer of placebo ($n = 16$) and *Agaricus sylvaticus* ($n = 16$) groups treated at the Proctology Outpatient Clinic, Hospital de Base do Distrito Federal, throughout the clinical follow-up. Placebo: $p = 0.14$ and $p = 0.19$ and *Agaricus sylvaticus*: $p = 0.16$ and $p = 0.05$, after three and six months, respectively. Student's *t*-test.

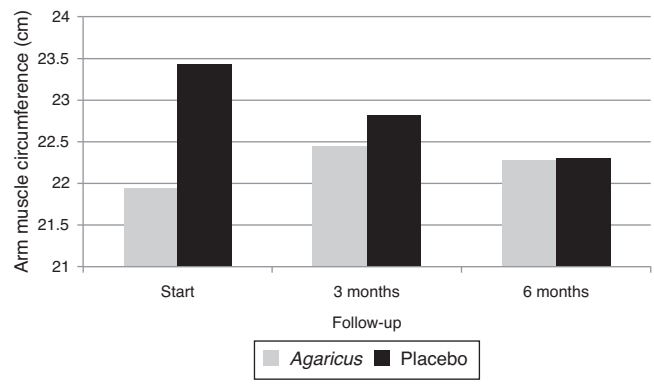

Fig. 4 – Arm muscle circumference (AMC) of women with colorectal cancer of placebo ($n = 16$) and *Agaricus sylvaticus* ($n = 16$) groups treated at the Proctology Outpatient Clinic, Hospital de Base do Distrito Federal, throughout the clinical follow-up. Placebo: $p = 0.14$ and $p = 0.03$ and *Agaricus sylvaticus*: $p = 0.10$ and $p = 0.22$, after three and six months, respectively. Student's *t*-test.

TSF. After an interval of three months, there was an increase to 20.43 ± 9.14 mm ($p = 0.14$) and after six months, a further increase to 20.42 ± 8.33 mm ($p = 0.19$). But these changes were not statistically significant (Fig. 3).

Within three months of supplementation, the *Agaricus sylvaticus* group showed a non-significant increase in TSF (from 21.41 ± 7.44 mm to 22.38 ± 5.95 mm; $p = 0.16$), followed after six months by a significant increase (from 21.41 ± 7.44 mm to 23.66 ± 5.62 mm, $p = 0.05$) (Fig. 3).

In the placebo group, after arm muscle circumference measurement, a non-significant decrease after three months (from 23.44 ± 4.42 cm to 22.82 ± 3.16 cm, $p = 0.14$) and a significant decrease after six months (from 23.44 ± 4.42 cm to 22.30 ± 3.29 cm, $p = 0.03$) were observed (Fig. 4).

These findings were not found in the *Agaricus sylvaticus* group, which increased its AMC after three (from 21.94 ± 2.61 m to 22.45 ± 2.44 cm, $p = 0.10$) and six (from 21, 94 ± 2.61 cm to 22.28 ± 2.65 cm, $p = 0.22$) months of supplementation, although these changes were not significant (Fig. 4).

As to the percentage of body fat, the placebo group presented initially a %BF of $36.33 \pm 8.44\%$. After three months, this variable had increased (from $36.33 \pm 8.44\%$ to $36.46 \pm 7.25\%$, $p = 0.44$) and in the sixth month, a new increase was again noted (from $36.33 \pm 8.44\%$ to $37.60 \pm 8.07\%$, $p = 0.19$). But these changes were not statistically significant (Fig. 5).

The *Agaricus sylvaticus* group presented an initial %BF of $36.88 \pm 7.33\%$. After three months, a non-significant increase to $37.88 \pm 6.60\%$ ($p = 0.09$) was found, and after six months a significant increase to $39.56 \pm 8.68\%$ ($p = 0.04$) was observed (Fig. 5).

Discussion

In this study, the sample was conveniently composed of 100% of women with colorectal cancer. Scientific evidence suggests that colorectal cancer is more prevalent in women, affecting more and more often the left colon.[11] In Brazil, the Instituto Nacional do Câncer (INCA) registry for 2012 estimated the occurrence of 14,180 new cases of colorectal cancer in men and of 15,960 cases in women, corresponding to an estimated risk of 15 new cases per 100,000 men and 16 new cases out of every 100,000 women.[12]

The mean age of our patients was 55 and 57 years for placebo and *Agaricus sylvaticus* groups, respectively. In these groups, the minimum and maximum ages were 32 and 77 years, respectively. According to Cozerattolini et al.,[13] more than 90% of colon and rectal cancers relate to individuals older than 50 years, and 75% of cases affect individuals without other risk factors, besides age.

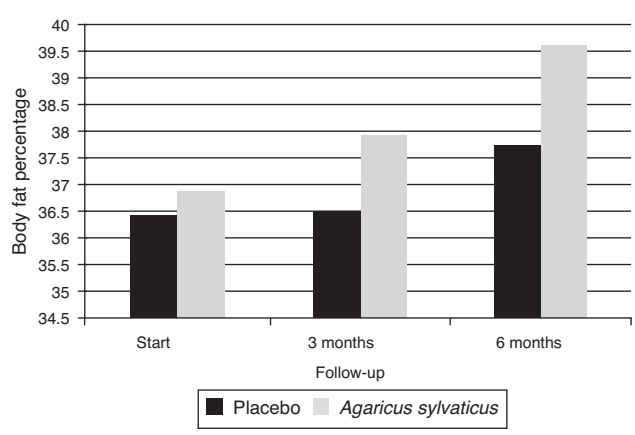

Fig. 5 – Body fat percentage (%BF) of women with colorectal cancer of placebo ($n = 16$) and *Agaricus sylvaticus* ($n = 16$) groups treated at the Proctology Outpatient Clinic, Hospital de Base do Distrito Federal, throughout the clinical follow-up. Placebo: $p = 0.44$ and $p = 0.19$ and *Agaricus sylvaticus*: $p = 0.09$ and $p = 0.04$, after three and six months, respectively. Student's *t*-test.

In this study, both placebo and *Agaricus sylvaticus* groups had an initial BMI within the normal weight range, with a tendency to be overweight. Scientific studies have shown a positive correlation between overweight, obesity and risk of developing several types of cancer, as well as in mortality from this disease. It is expected that the probable mechanism is interconnected with hyperinsulinemia and with a high level of insulin-dependent growth factor (IGF-1) and of those proteins that bind to IGF-1, as well as with the practice of diets characterized by too much energy consumption.[13]

There is a higher correlation between excess weight and risk of colorectal cancer, in which the abdominal or central distribution of body fat is the main component of increasing this risk, as this occurrence is robustly linked to insulin resistance and hyperinsulinemia.[13] However, in this study, visceral fat was not evaluated.

Clinical and experimental studies show that the diet supplemented with *Agaricus sylvaticus* and other fungi promotes positive effects with respect to nutritional, medicinal and pharmacological effects, and that these supplements can be used as adjuvants in cancer treatment.[14] Medicinal fungi exert anabolic effects, because they contain all the essential amino acids, plus immunonutrients like arginine and glutamine that, in times of metabolic stress, become conditionally essential, contributing to improvements in nitrogen balance.[1,15]

In this study, the *Agaricus sylvaticus* group obtained better anthropometric results (BMI, AC, TSF, %BF) *versus* placebo, including in relation to lean body mass, despite no significant finding. Scientific evidence suggests that medicinal fungi have bioactive compounds able to prevent the muscle protein catabolism commonly present in these patients, explaining in part the results observed.[15–17]

The mechanisms of action of existing bioactive compounds in fungi are not yet fully explained in the literature, but scientific studies suggest that these substances can modulate carcinogenesis, not only in the early stages, but also in advanced phases in the progression of the disease, especially by stimulating the immune system.[14]

We found no scientific papers in the literature that evaluated anthropometry and/or the nutritional status of cancer patients after dietary supplementation with medicinal fungi, including those of the Agaricaceae family, species *Agaricus sylvaticus*. However, clinical studies show that medicinal fungi are able to modulate the metabolism of carbohydrates, proteins and lipids, besides exerting beneficial effects on the hematopoietic, immune and gastrointestinal systems, with positive repercussions on the quality of life of these patients.[15–24]

Major metabolic alterations induced by advanced tumors include glucose intolerance, decreased insulin secretion, peripheral insulin resistance, increased synthesis and glucose turnover, increased activity of the Cori cycle, increased protein turnover, increased hepatic protein synthesis, increased muscle protein catabolism, reduced plasma concentration of branched chain amino acids, depletion of lipid deposits, increased lipolysis, increased glycerol and free fatty acid turnover, reduced lipogenesis and hyperlipidemia.[4,5,15,16,20,21,23]

The depletion of adipose tissue is responsible, in large part, by the weight loss observed in cancer patients. This is due to the different changes in fatty acid metabolism and also to the occurrence of lipolysis, increased lipid oxidation, reduction of lipogenesis and of lipoprotein lipase activity, and increased release of lipolytic tumor factors and hormone-sensitive lipase, resulting in hyperlipidemia.[15,16,19,20,22]

The beneficial effects of these fungi have been demonstrated, with inhibition of anti-tumor activity and proliferation of cancer cells, expansion of natural killer cell function and of other immunological parameters, such as the secretion of immunoglobulins IgA, IgM and IgE, and a progression of monocyte and macrophage functions.[5,16,21,22]

It is noteworthy that, in addition to high biological value proteins and of immunomodulator amino acids (such as arginine and glutamine) that help in muscle protein anabolism of cancer patients, other substances present in medicinal fungi stand out: glucans, proteoglucans, lectins, ergosterol and triterpenes – all with the ability of modulating the various metabolic and immune actions in these patients.[5,15,16,22,23]

Conclusion

Our results suggest that dietary supplementation with *Agaricus sylvaticus* fungus has the ability to bring benefit for anthropometric parameters of women with colorectal cancer. However, controlled and randomized clinical trials, in addition to those performed in this study, are needed to elucidate the mechanisms of action of the bioactive principles present in *Agaricus sylvaticus*, as well as other medical conditions that could benefit through this supplementation.

Conflicts of interest

The authors declare no conflicts of interest.

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