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Effects of a low-fat diet with antioxidant supplementation on biochemical markers of multiple sclerosis long-term care residents

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Abstract

Introduction: **Multiple sclerosis (MS) treatment options are primarily limited to immunomodulatory therapies in MS non-progressive forms. Nutrition intervention studies suggest that diet may be considered as a complementary treatment to control disease progression. Therefore, dietary intervention may help to improve wellness and ameliorate symptoms of MS patients.**

Objectives: **To assess the effect of a low-fat diet with antioxidant supplementation on biochemical markers of institutionalized patients with progressive forms of multiple sclerosis.**

Methods: **A randomized prospective placebo-controlled study involving 9 participants, 5 of them assigned to the intervention group (low-fat diet and antioxidant supplementation) and the other 4 to the placebo group (low-fat diet). The effect of the dietary intervention, involving diet modification and antioxidant supplementation, was examined for 42 days by measuring anthropometric, biochemical parameters and oxidative stress markers in blood at baseline (day 0), intermediate (day 15) and end (day 42) stages of the treatment.**

Results: **The intervention group obtained C reactive protein levels significantly lower than those observed in the corresponding placebo group at the end of the study. Oxidative stress and inflammatory markers isoprostane 8-iso-PGF2α and interleukine IL-6 values also diminished after dietary intervention in the intervention group. Catalase activity increased significantly in the intervention group prior antioxidant supplementation. No significant differences were observed in other oxidative stress markers.**

Conclusions: **The results suggest that diet and dietary supplements are involved in cell metabolism modulation and MS-related inflammatory processes. Consequently, low fat diets and antioxidant supplements may be used as**

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EFECTO DE UNA DIETA BAJA EN GRASAS CON SUPLEMENTACIÓN DE ANTIOXIDANTES EN LOS MARCADORES BIOQUÍMICOS DE RESIDENTES DE LARGA ESTANCIA CON ESCLEROSIS MÚLTIPLE

Resumen

Introducción: **Las posibilidades de tratamiento de la esclerosis múltiple (EM) se encuentran limitadas principalmente a terapias con inmumoduladores en las formas no progresivas de EM. Los estudios de intervención nutricional sugieren que la dieta puede considerarse como un tratamiento alternativo para controlar la progresión de la enfermedad. Por esta razón, las intervenciones en la dieta pueden ayudar a mejorar el bienestar y mejorar los síntomas de los pacientes con EM.**

Objetivos: **Valorar el efecto de una dieta pobre en grasas con suplementación de antioxidantes en los marcadores bioquímicos de pacientes institucionalizados que presentan formas progresivas de EM.**

Métodos: **Se realizó un estudio prospectivo aleatorizado controlado por placebo con 9 participantes, 5 de los cuales se asignan al grupo de intervención (dieta baja en grasas y suplementación antioxidante) y los 4 restantes al grupo placebo (dieta baja en grasas). Se evaluó el efecto de la intervención dietética que supone modificación de la dieta e introducción de antioxidantes durante 42 días mediante valoraciones de parámetros antropométricos y bioquímicos y marcadores del estrés oxidativo en sangre y orina en las etapas inicial (día 0), intermedia (día 15) y final (día 42) del tratamiento.**

Resultados: **Se obtuvieron niveles de proteína C reactiva significativamente inferiores en el grupo de intervención con respecto al grupo placebo al final del estudio. Los marcadores de estrés oxidativo e inflamación: isoprostanos 8-iso-PGF2α e interleucina IL-6 también disminuyeron en el grupo de intervención después de la intervención dietética. La actividad de la enzima catalasa aumentó de forma significativa en el grupo de intervención antes de la suplementación con antioxidantes. No se observaron diferencias significativas en otros marcadores de estrés oxidativo.**

Conclusiones: **Los resultados obtenidos sugieren que la dieta y los suplementos dietéticos están involucrados en la modulación del metabolismo celular y los procesos de inflamación de la EM. En consecuencia, las dietas bajas**

complementary therapies for treatment of multiple sclerosis.

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Palabras clave: *Dieta baja en grasas. Suplementos antioxidantes. Verbascósido. Esclerosis múltiple.*

Introduction

Multiple sclerosis (MS) is a complex multi-factorial neurodegenerative disease in which inflammation, demyelination and axonal damage are the major pathophysiological processes. Although the cause of MS is not yet fully understood, it is generally accepted that demyelination results on an autoimmune dysregulation mediated by the activation of inflammatory lymphocytes (CD4+T helper cells, Th17 cells and B lymphocytes)[1,2]. Focal infiltration of T cells, macrophages, microglial cells and other immune mediators into the blood-brain barrier initiate neuroaxonal loss and tissue injury[3-5]. Recent pathological patterns suggest that mitochondrial dysfunction and subsequent energy failure may also contribute to explain MS pathogenesis involving oligodendrocyte apoptosis and breakdown of the myelin sheath[2,6]. As a result, multifocal white matter and cortical lesions generated by myelin degradation drive to increasing disability levels including walking impairment, fatigue, imbalance, upper extremity dysfunction and cognitive decline.

MS disease course is heterogeneous and depends on the clinical presentation. There are three main MS types ranging from the relapsing-remitting MS (RRMS) disease form to the primary progressive MS (PPMS) and secondary progressive MS (SPMS) clinical subtypes. Most of MS patients begin with symptoms of neurological worsening followed by partial recovery typical of the RRMS form but within a period of 10-20 years from onset they enter a phase of slow and relentless progression called secondary progressive. PPMS disease course progress with slow relapses and is present only in a minority of patients (15%).

Because of MS heterogeneity and patient response variability, the use of pharmacological therapies, in particular immunomodulatory agents (interferon-β, glatiramer acetate), monoclonal antibodies (natalizumab) and chemotherapy agents (mitoxantrone), has been ineffective in progressive forms of MS and only partially effective in relapsing-remitting MS[7,8]. Therefore, the development of new therapies for controlling either disease or symptoms progression is needed, especially in MS progressive forms.

A number of MS patients (33-70%)[7] use complementary or alternative medicine in the belief of improving disease outcomes. From this angle, nutritional interventions can be considered a very promising approach to complement conventional MS treatments. Studies on the role of diet and dietary supplements such as polyunsaturated fatty acids (PUFA), vitamins, micronutrients and antioxidants in MS process have been reported in recent years[2,8-11]. Quercetin and interferon-beta have been reported to modulate immune responses in peripheral blood mononuclear cells isolated from sclerosis patients[12]. Investigation on PUFAs suggest the potential benefits of omega-3 and omega-6 fatty acids as anti-inflammatory and neuroprotective agents[13,14]. Dietary supplementation with linoleic/lipoic acid, gingko biloba, selenium and Coenzyme Q has also being tested as antioxidant therapies with unequal results.

Likewise, interventions on fat content, nutrients and dietary intake are very limited. Therefore, further well-designed randomized placebo-controlled trials are needed to support clinical and experimental data on the beneficial effects of dietary interventions over disease course and progression.

In this study we present a nutritional intervention on MS patients from a long term care facility consisting on reducing dietary fat content while supplementing with an antioxidant/anti-inflammatory compound. The aim of the study was the investigation of the effectiveness of diet and dietary supplementation on biochemical markers of patients with progressive forms of multiple sclerosis. The analysis of biochemical parameters included inflammatory and oxidative markers i.e. oxidation enzymes, cytokines Il-6 and isoprostaglandines in blood and urine samples.

Methods

Subjects

A group of 9 MS patients with secondary progressive MS (n = 4 for diet intervention and n = 5 for diet intervention and antioxidant supplementation) was recruited from a long term care centre. Exclusion criteria included the use of food therapeutic products. Differences between groups in clinical and anthropometric characteristics were not significant. All patients have extended disability status scale (EDSS) scores higher than 6.5, which can be categorized as severe disability. The study was approved by the Ethical Committee of the University of León and participants were informed and provided written consent prior study inclusion.

Protocol

The protocol was in accordance with the Helsinki declaration for Research on human beings. Anthropometric parameters were collected at day 0, day 14 and day 42 of the study.

Diet intervention consisted on the replacement of either the first or second course at lunch by prepared foods from Campofrio Food Group. Prepared foods included triturated meals or processed meat products. The diet was adapted by a dietician according to the specific requirements of each patient (caloric expenditure: 1500 Kcal, type of mastication/chewing diet). Prepared foods were given five days per week during 42 days to all participants. Prepare foods' fat content did not exceed 30% of total daily calories.

Food intake of prepared products was recorded over the whole period of the study. The dietary assessment from day 0 to 14 was used to categorize the participants into two treatment groups. Patients with less records of food items consumed (n = 4) were assigned to the group just with diet intervention while participants with complete daily food records (n = 5) were included into the group involving diet modification and antioxidant supplementation. The vegetal extract containing Lipia citriadora (PLX®) was used as anti-inflammatory/antioxidant supplement. The intervention group DIETAO received 200 mg/day of PLX® powder (10% verbascoside w/w) added to the prepared food use as replacement product from day 14 to day 42 of the study (5 days a week).

Assays: Blood and urine sampling

Blood samples were collected after overnight fasting at day 0, 14 and 42 of the study.

Biochemical serum parameters including glucose, triglycerides, cholesterol, creatinine, ferritin, urea, insulin homeostatic model assessment (HOMA) (data not shown) and C reactive protein (CRP) were analysed by a clinical hematology laboratory according to international standards.

Enzyme oxidation activity was measured in our laboratory from another blood sample by the following methods. Total antioxidant status (TAS) and catalase (CAT) activity were evaluated by the Re (1999) and Aebi (1984) methods respectively[15-17]. Glutathione peroxidase (GPx) was determined by the Gunzler and Flohé (1984) method[18,19] and superoxide dismutase (SOD) activity measurements were performed using the Oberley and Spitz technique (1984)[20].

The levels of IL-6 levels in serum were determined using an immunoassay kit provided by Bionova científica S.L (Madrid, Spain) according to manufacturer's instruction. The estimation of isoprostanes in urine was developed by using an immunoassay commercial kit Bionova científica S.L (Madrid, Spain) to determine 8-isoPGF2α following manufacture's indication. Urine samples were obtained from participants at baseline, intermediate and end stages of the study by either drip collector or urinary intermittent catheterization.

Statistical Analysis

Data from three separated experiments corresponding to the different stages of the study were assessed. Statistical analysis was performed using the SPSS 19.0 programme (SPSS Inc., Chicago, IL, USA). Results were expressed as mean values ± SD (standard deviation). Values of $p < 0.05$ were considered statistically significant. As results followed a normal distribution, the Student test was used to estimate the effect of diet intervention and antioxidant supplementation.

Results

All nine subjects completed the study. Baseline demographic and clinical characteristics were similar in both groups (Table I). Pharmacological and symptomatic treatment of participants did not differ between groups and remain constant from baseline to the end of the study. Specific requirements were taken into account depending on the type of diet (hypocaloric,

Table I
Demographic and anthropometric characteristics of participants[a]

	Day 0		*Day 14*		*Day 42*	
	Placebo group n = 4	*Supplemented group n = 5*	*Placebo group n = 4*	*Supplemented group n = 5*	*Placebo group n = 4*	*Supplemented group n = 5*
Mean age (years)	55.75 (4.72)	56.2 (7.19)				
Gender (m:f)	1:3	2:3				
Height (cm)	156.5 (5.19)	167.8 (15.04)				
Weight (kg)	61.87 (9.93)	66.7 (11.05)	59.07(8.79)	71.04 (13.74)	59.6 (9.55)	71.82 (13.99)
Body mass Index	25.43 (2.88)	24.76 (2.43)	24.1 (3.26)	25.08 (2.28)	24.3 (3.51)	25.35 (2.33)
Disease duration (years)	16 (4.24)	16 (6.19)				

[a]Data are expressed as mean (standard deviation)

puréed or easy mastication) in order to adapt total daily calories, food texture and fat content. Patient characteristics and anthropometric values including height, weight and Body-mass Index (BMI) at day, 0, 14 and 42 are summarized in Table I. No significant variations in weight and body mass were seen between groups along the trial nor over/under-weight deviations as response to diet intervention.

Most of the biochemical parameters were within ordinary ranges although comparison between groups yielded some differences (see Table II). Glucose, cholesterol, lipids, protein (albumin, prealbumin) and mineral concentrations (data not shown) within groups did not change significantly from baseline to the end of the trial.

The most striking result was the decrease of mean C reactive protein levels in the supplemented group. CRP concentration reached a difference value of 6.40 mg/L (SD 13.31) after supplementation while an increment of 9.125 mg/L (SD 14.15, $p < 0.05$) was observed in the placebo group at the end of the study.

Slight differences were found in antioxidant enzyme activities from patients consuming the antioxidant supplement compared to the placebo group (Table III). Total antioxidant status (TAS) values in serum remain equivalent throughout the study and the only statistical significant difference between groups was observed at the intermediate stage (day 14) of the study. Glutathione peroxidase levels were also within normal ranges although a slight decrease was seen from diet intervention (day 0) to the end of the study in either supplemented (difference -1.91, SD 1.63) or placebo (difference -1.72, SD 1.04) groups. SOD activity increase similarly in both groups (difference 1.58, SD 2.81 and difference 1.84, SD 1.67) along the trial (from day 0 to day 42). The most relevant differences were found for catalase levels. The increase in catalase antioxidant activity was significantly higher in the supplemented group (difference value of 35.09, SD 63.26) after diet intervention (day 14) in comparison with the placebo group (difference value 1.28, SD 73.12).

The results obtained from the analysis of other oxidative markers were in agreement with this trend (Fig. 1 a). The levels of IL-6 in serum decreased in the supplemented group prior antioxidant supplementation (difference 3.26, SD 6.2 $p < 0.05$) and remain stable during the last stage of the study (difference 0.27, SD 1.13 $p < 0.05$). On the contrary, the mean value of IL-6 in the placebo group increased from baseline to the

Table II
Changes on mean scores of biochemical parameters throughout the study[a]

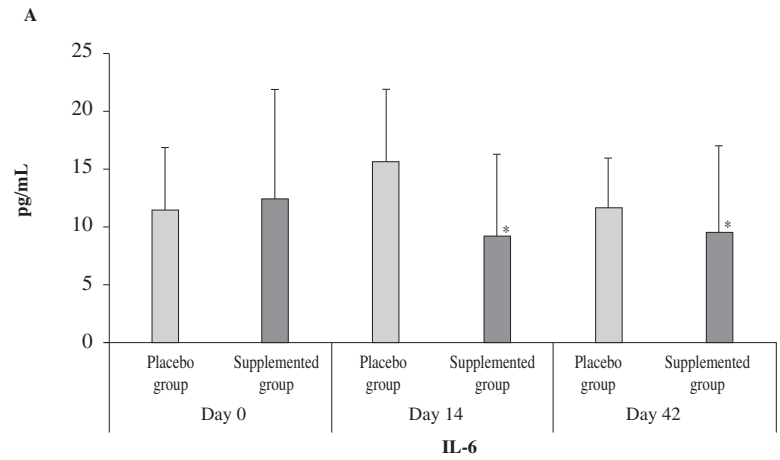
	Day 0		*Day 14*		*Day 42*	
	Placebo group n = 4	*Supplemented group n = 5*	*Placebo group n = 4*	*Supplemented group n = 5*	*Placebo group n = 4*	*Supplemented group n = 5*
Glucose (g/dL)	83.5 (10.37)	75.4 (6.80)	82 (11.16)	70.8 (9.23)	89 (7.87)	76.75 (6.65)*
Cholesterol total (mg/dL)	172.5 (42.88)	236.4 (88.20)	180.25 (48.43)*	232.6 (69.41)*	175 (48)*	237.8 (84.64)*
Triglycerides (mg/dL)	102.75 (41.72)	164.2 (109.91)	99.5 (26.85) ‡	151.8 (84.83) ‡	113.5 (44)	147.4 (89.82)
Lipids total (mg/dL)	534.25 (147.22)	755.4 (324.13)	550.5 (147.64)*	733.4 (229.25)*	551.25 (160)*	742.2 (300.74)*
Albumine (g/L)	3.7825 (0.49)	4.162 (0.29)	3.885 (0.33)*	4.066 (0.29)*	3.8125 (0.32)	4.136 (0.31)
Retinol Binding Protein (mg/dL)	3.775 (2.58)	4.725 (0.53)	3.4 (1.94)*	5.06 (1.07)*	3.5 (2)*	5.54 (1.47)*
C reactive protein CRP (mg/L)	16.325 (19.11)	4.08 (3.36)	22.52 (25.76)*	9.88 (13.06)	19.11 (33)*	3.48 (1.46)

[a]Data are expressed as mean (standard deviation).
*Intra-group values ($p < 0.05$) are significantly different according to the Student test after intervention.
‡Inter-group values ($p < 0.05$) are significantly different according to the Student test.

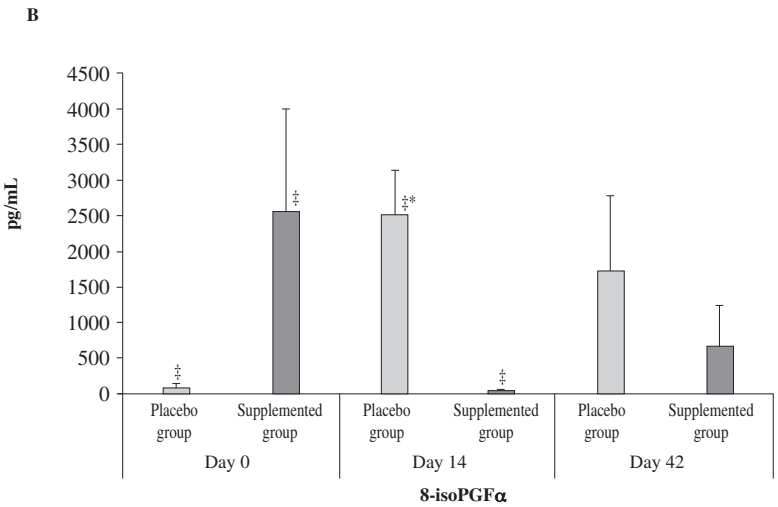
Table III
Effect of extract supplementation on enzyme oxidation activity

		Day 0	*Day 14*	*Day 42*
TAS (U/mg protein)	Placebo group	0.82 (0.28)	0.70 (0.21)‡	0.82 (0.12)
	Supplemented group	0.84 (0.17)	0.74 (0.16)‡	0.90 (0.20)
SOD (U/mg protein)	Placebo group	3.86 (1.62)	4.17 (2.14)	5.44 (1.55)
	Supplemented group	3.38 (1.1)	3.65 (1.12)	4.94 (1.59)
GPx (U/mg protein)	Placebo group	2.86 (0.48)‡	0.87 (0.62)	0.95 (0.67)‡
	Supplemented group	2.53 (1.55)‡	0.76 (0.33)	0.82 (0.20)‡
Cat (U/mg protein)	Placebo group	97.14 (45.38)	98.42 (20.59)	105.64 (51.76)
	Supplemented group	69.83 (26.24)	104.92 (49.17)	98.72 (56.17)

[a]Data are expressed as mean (standard deviation).
*Intra-group values ($p < 0.05$) are significantly different according to the Student test after intervention.
‡Inter-group values ($p < 0.05$) are significantly different according to the Student test.



*Intra-group values ($p < 0.05$) are significantly different according to the Student test after intervention
‡Inter-group values ($p < 0.05$) are significantly different according to the Student test



[a]Data are expressed as mean (standard deviation)
*Intra-group values ($p < 0.05$) are significantly different according to the Student test after intervention
‡Inter-group values ($p < 0.05$) are significantly different according to the Student test

Fig. 1.—Representation of mean scores (SD) for: a) IL-6 and b) 8-isoPGF2α values.

intermediate stage (difference 4.12, SD 4.58) and diminished subsequently at the end of the study (difference -3.87, SD 4.37).

The same response was observed for measurements of isoprostanes as lipid peroxidation markers in urine. For the supplemented group the decrease in mean values between the baseline and the end of the study was 1889.50 pg/mL (SD 1115.01) while an increment of 1634.66 pg/mL (SD 2109.36) was observed for the placebo group throughout the same period (Fig. 1 b).

Discussion

In spite of recent advances in the development of immunomodulator drugs, pharmacological therapies have been proven ineffective in severe presentations of multiple sclerosis including secondary progressive MS. At present, the performance of therapeutic interventions is primarily focused on ameliorating symptoms in order to improve the patient's quality of life. Amongst complementary treatments nutrition has been considered as a decisive factor to control symptoms and enhance wellness of MS patients. Although no special diets are associated with MS, the impact of diet and dietary supplements on the course of progressive forms of the disease has been studied during the last years[5].

In the present study our findings in biochemical parameters and oxidative stress markers after diet intervention in MS patients are reported. The impact of prepared food products as substitutes of high fat content courses revealed differences in antioxidant and anti-inflammatory parameters between treatment groups. The most remarkable result is the increase of catalase activity and the reduction in CPR reactive values in the antioxidant supplemented group.

Previous studies have evaluated the effect of diets with low saturated fat content on patients with relap-

sing-remitting MS. This is the first study to prove the influence of a low fat diet in persons with progressive presentations of MS. The reduction of fat content was first studied by Swank in the 1950's by comparing different levels of fat and oil consumption with the development of disability and the frequency of mortality in MS patients along 35 years with a non-randomized retrospective trial[21]. Swank suggested that following a diet with low fat content (average 15 g/day) in both total and saturated fats provided better outcomes in terms of disability and disease activity.

Another trial has obtained moderate benefits on physical and mental health of RRMS patients by assessing the influence of a low fat dietary intervention with ω3 polyunsaturated fatty acid supplementation8. However, the effect of ω3 polyunsaturated fatty acid supplementation is controversial. Some studies have associated ω3 fatty acid supplementation with decreased matrix metalloproteinase-9 (MMP-9) levels and potential immune-modulator action while others have not detected beneficial effects when compared with interferon β treatment[11-13].

In our study fat content was diminished in more than 50% by introducing courses low in total and saturated fats (data not shown). As mentioned above, patients who followed inconstantly diet modifications were included in the placebo group. Food daily intake records of these participants indicated less consume of prepared food products in a range of 40-75% when compared with the supplemented group. Lipid levels were not affected and remain constant throughout the study, although several differences were found after diet modification in enzyme oxidation activity and inflammatory markers. The most remarkable changes were observed in the supplemented group after diet intervention in catalase activity, IL-6 and 8-isoPGF2α values.

The increase in catalase activity prior supplementation may respond to the balanced effect of fat composition on the diet. SOD levels also increased slightly in both groups after diet intervention. The production of reactive oxygen species is increased in inflammatory and demyelinating diseases like MS as a response to the failure of cellular detoxification and antioxidant mechanisms[6,22]. Since the catalase enzyme remove hydrogen peroxyde produced during inflammation, the increase of catalase activity may explain the reactivation of the detoxifying process after reducing dietary fat.

Similar effects were observed for IL-6 and 8-isoPGF2α in serum and urine samples after diet intervention. Cytokines are implicated in MS autoimmune response and neuronal dysfunction initiated by T-cell and macrophage infiltration[23]. In particular, IL-6 induces differentiation of TH17 and B cells[23,24] and immunoglobulin production resulting on a good biomarker of inflammatory disease activity. Although the slight decrease observed in the supplemented group prior antioxidant supplementation is not statistically significant it suggests that a low fat diet may contribute to reduce blood levels of IL-6 and other pro-inflammatory markers[25].

The assessment of lipid peroxidation markers revealed a significant variation of 8-isoPGF2α in the supplemented group. The effect of verbascoside, one of the components contained on the plant extract supplementation, in phospholipid membranes shows its potential as a lipid antioxidative agent[26]. Nevertheless, changes in isoprostanes urine levels were attained prior antioxidant supplementation. The presence of lipid peroxidation products in body fluids and its relationship with MS and disability progression have been previously reported[27]. The comparison of isoprostanes levels between MS patients and controls suggest that 8-epi-prostaglandin (PG)-P2α may be used as a valuable non-invasive marker of oxidative stress. In our study the significant decrease of 8-isoPGF2 after reassessing the fat balance on the diet may explain the impact of fatty acid composition on the inflammatory and oxidative process of multiple sclerosis[8,13].

C reactive protein was the only inflammatory marker that decreased after antioxidant supplementation. The supplemented group presented higher CRP levels in serum prior supplementation that subsequently diminished at the end of the study. This drop may respond to the effect of the anti-inflammatory properties of verbascoside. The impact of lemon verbena extracts containing verbascoside as a complementary treatment to inflammatory response has been investigated in joint management, resistance exercise and inflammatory bowel disease[28-30]. The study of immune cell membrane fatty acids in MS patients also suggests a correlation between CRP and phospholipid composition of cell membranes[31]. In addition, verbascoside antioxidative effect in phospholipid membrane structures have been previously reported[32]. Therefore it may be considered that verbascoside supplementation may contribute to modulate the inflammatory response and oxidative damage of demyelinating and chronic inflammatory diseases.

Although sample size limits the statistical significance of this study as well as the interpretation of results, the comparison between placebo and supplemented groups allows the assessment of relevant trends and set the basis for further investigation. The examination of the therapeutical effects of a low fat diet in relapsing and progressive forms of MS may be an interesting approach to complement the limited range of treatments available. Likewise, the impact of dietary supplements on inflammation may be improved by enlarging the size of randomized, double-blind placebo-controlled studies. The effectiveness of complementary and alternative therapies should also rely on the development of feasible non-invasive markers that allow the quantification of disability activity and the implementation of disability progression scales in order to improve the quality of life of MS patients.

Conclusions

This paper reports the effect of a low fat dietary intervention with antioxidant supplementation in 9 MS

institutionalized patients for 42 days. The assessment of oxidative and inflammatory markers in urine and serum samples show variations in 8-iso-PGF2α and IL-6 levels after dietary intervention. Catalase activity was also affected by the reduction of fat content on the diet whereas C reactive protein values diminished significantly in the intervention group after antioxidant supplementation. The influence of fat consumption and dietary supplements on the activity and progression of multiple sclerosis is still under discussion. Therefore, further studies are needed to confirm the effect of nutritional interventions in multiple sclerosis.

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