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Basic nutritional investigation

Role of dietary α- and γ-tocopherol from Rosa mosqueta oil in the prevention of alterations induced by high-fat diet in a murine model

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ABSTRACT

Objective: The aim of this study was to evaluate the contribution of tocopherols present in Rosa mosqueta oil (RM) in the prevention of high-fat diet (HFD)-induced alterations.
Methods: Male C57 BL/6 J mice (n = 9/group) were fed for 12 wk and divided into four groups: control (CD; 10% kcal fat, 20% kcal protein, 70% kcal carbohydrates); HFD (60% as fat, 20% kcal protein, 20% kcal carbohydrates); HFD + RM (0.01 mL/g body weight/d); and HFD + RM$^-$ without tocopherols (0.01 mL/g body weight/d). Parameters of obesity, liver steatosis (histology, triacylglycerols content), inflammation (adipose NLRP3 inflammasome, tumor necrosis factor-α and interleukin-1 β expression, hepatic nuclear factor-κB) and oxidative stress (hepatic Nrf2 activation, carbonylated proteins) were evaluated.
Results: Liver steatosis, inflammatory, and oxidative stress parameters were significantly ($P < 0.05$) increased in the HFD + RM$^-$ compared with the HFD + RM, with no differences between HFD and HFD + RM$^-$.
Conclusion: The present study suggests that α- and γ-tocopherols from RM may have an important role in the prevention of alterations induced by HFD.

Introduction

Obesity, considered one of the most important chronic diseases, has become a public health problem in the Western world because of its high prevalence and its association with multiple comorbidities. The pathophysiology of obesity is complex due to several genetic, metabolic, endocrinologic, and environmental factors involved in its origin and development. However, an overconsumption of lipids and carbohydrates may explain most of the prevalence of this clinical condition [1]. Obesity is strongly associated with metabolic syndrome (MetS), which encompasses a cluster of risk factors that predispose to the development of cardiovascular disease, type 2 diabetes mellitus (T2DM), and insulin resistance (IR) [2]. Obesity is associated with adipose tissue hypertrophy and hyperplasia; and increases in nuclear factor (NF)-κB activity, proinflammatory cytokines, recruitment of monocyte/macrophages, and *NLRP3* inflammasome activation, which in turn alters the normal functioning of peripheral tissues [3,4]. It also has been established that obesity and IR are strongly associated with the chronic oxidative stress development associated with depletion in antioxidant systems such as nuclear factor (erythroid-derived 2)-like 2 (Nrf2), producing a long-term imbalance in physiological homeostasis [5,6].

Scientific evidence supports the dietary administration of omega-3 fatty acids (ω-3) for the treatment of MetS [7]. Rosa mosqueta oil (*Rosa rubiginosa*, RM) is a nutritional source of α-linolenic acid (18:3 ω-3, ALA) [8,9], and also has a high vitamin E content as α- and γ-tocopherol (in a 1:5 ratio) compared with other healthy oils [10]. In previous studies, we demonstrated that RM administration prevents the metabolic disruptions induced by a high-fat diet (HFD) in mice, protecting against HFD-induced hepatic steatosis, IR, inflammation, and oxidative stress [11–13]. Due to their lipophilic character, α- and γ-tocopherols fulfill their antioxidant function mainly attached to lipid membranes,

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decreasing the lipid peroxidation of unsaturated fatty acids by the scavenging of free radicals [14,15]. Previous studies have indicated that α- and γ-tocopherol administration has therapeutic effects in the treatment of the MetS. For example, α-tocopherol supplementation in diabetic rats decreased intrahepatic triacylglycerol (TG) accumulation and lipoperoxidation and reversed the abnormal increase in both glycemia and oxidative stress [16]. On the other hand, coadministration of γ-tocopherol and docosahexaenoic acid (DHA) decreased inflammation and oxidative stress in patients [17], whereas coadministration of spironolactone and vitamin E significantly decreased homeostatic model assessment for assessing insulin resistance (HOMA-IR) in obese patients [18], and tocopherol (α- and γ-) administration decreased oxidative stress and inflammation in obese mice with lipopolysaccharide (LPS)-induced steatohepatitis [19].

The aim of this study was to evaluate the effect of RM-lacking α- and γ-tocopherols (RM^{-}) on the prevention of HFD-induced MetS. For this purpose, the parameters of obesity, liver steatosis, inflammation, and oxidative stress were evaluated.

Materials and methods

Ethics statement

Experimental animal protocols and procedures complied with the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, NIH Publication 6-23, revised 1985) and were approved by the Bioethics Committee for Research in Animals, Faculty of Medicine, University of Chile (CBA 0744 FMUCH).

Animal preparation and supplementation with RM

Weaned male C57 BL/6 J mice weighing 12 to 14 g (Animal Facility at the Faculty of Medicine, University of Chile) were randomly divided into four groups and fed for 12 wk as follows: control diet (CD) containing 10% kcal fat, 20% kcal protein, and 70% kcal carbohydrate (D12450 B, Research Diets, New Brunswick, NJ, USA); HFD containing 60% kcal fat, 20% kcal protein, and 20% kcal carbohydrate (D12492, Research Diets); HFD plus oral RM (HFD + RM, 0.01 mL/g body weight [BW]/d) (Coesam, Chile), and HFD plus oral RM depleted of tocopherols content (HFD + RM^{-}, 0.01 mL/g BW/d). After 12 wk, the animals were fasted (6–8 h) and then anesthetized with Zoletil (20–40 mg/kg). Supplementary Table S1 shows the nutritional composition of the diets. Table 1 shows fatty acid and tocopherol composition of RM.

Table 1
Fatty acids and tocopherols composition of Rosa mosqueta oil

Fatty acid	g/100 g FAME
SFAs	
Palmitic acid (C16:0)	3.5
Stearic acid (C18:0)	1.8
Eicosanoic acid (C20:0)	0.75
Docosanoic acid (C22:0)	0.16
Total SFAs	6.3
MUFAs	
Palmitoleic acid (C16:1)	0.12
Oleic acid (C18:1)	14
Eicosaenoic acid (C20:1)	0.35
Total MUFAs	15
PUFAs	
Linoleic acid (C18:2 ω-6)	43
α-linolenic acid (C18:3 ω-3)	34
Total PUFA	77
ω-6/ω-3 fatty acid ratio	1.3
α-tocopherol	74
γ-tocopherol	359

FAME, fatty acid methyl esters; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

Tissue and blood samples

Liver and visceral adipose tissue (VAT) samples were weighed, frozen in liquid nitrogen, and stored at –80°C, or fixed in phosphate-buffered formalin and embedded in paraffin for further histology and immunohistochemistry analysis. Blood samples were taken by cardiac puncture and then centrifuged, and serum was stored at –20°C.

Biochemical determinations

IR was estimated by HOMA-IR [fasting insulin (μUI/mL) × fasting glucose (mg/dL)/405] [20], whereas cholesterol (mg/100 mL) levels were measured using specific diagnostic kits (Wiener Lab, Rosario, Argentina).

Liver histology and TG content assessment

Liver tissue slides were stained with hematoxylin-eosin and assessed by optical microscopy (Olympus CX31, Tokyo, Japan) at 400× for morphology analysis in a blind fashion. Both steatosis and inflammation were graded as absent, mild, moderate, or severe. Liver TG content was assessed by modified Bligh and Dyer method [21]. Results were expressed as g fat/100 g of liver.

Digital image analysis of adipose tissue sections

Adipose tissue sections were stained with hematoxylin-eosin and assessed by optical microscopy at 400×. Ten random images per slide were taken using Micrometrics SE Premium software (Accu Scope Inc., Commack, NY, USA). Fiji software (ImageJ v.1.51 n; National Institutes of Health, Bethesda, MD, USA) was used for measuring the individual adipocyte's cross-section area in each image.

Immunohistochemistry studies

Immunostaining for NF-κB and Nrf2 in liver sections was performed after deparaffinization, rehydration, and antigen retrieval with EDTA. Endogen peroxidase activity was blocked, followed by blocking of unspecific bounds with horse normal serum. Incubation with primary antibody anti NF-κB (PA1-30408; rabbit IgG, Thermo Fisher Scientific, Waltham, MA, USA) and anti-Nrf2 (PA5-27882; rabbit IgG, Thermo Fisher Scientific) was performed overnight at 4°C. Secondary antibodies were revealed using the ImmPACT DAB kit (Vector Labs, Burlingame, CA, USA) following manufacturer's recommendations. Mayer's Hematoxylin was used for nuclear contrast (modified solution according Lillie, ScyTek Laboratories, Logan, UT, USA). Analysis of positive nuclei was performed under light microscope in a blind fashion in 10 adjacent (400×) per slide.

qPCR assay for mRNA expression

Tumor necrosis factor (TNF)-α, interleukin (IL)-1 β, *NLRP3*, apoptosis-associated Speck-like protein (ASC), and caspase-1 mRNA expression in VAT was performed by real-time quantitative polymerase chain reaction. Nucleotide sequences for sense and antisense primers used in this study were as follows: 5′-TATGGCTCAGGGTCCAACTC-′3 and 3′-GCTCCAGTGAATTCGGAAAG-′5 for *TNF-α*; 5′-GGGAAACAACAGTGGTCAGG-′3 and 3′-GAGCT GTCTGCTCATTCACG-′5 for *IL-1 β*; 5′-TGCAACCTCCAGAAACTGTG-′3 and 5′-AGAACCAATGCGAGATCCTG-′3 for *NLRP3*; 5′-TAAA CACGGGTGCATGTCTG-′3 and 5′-GAAACAGAATGGCCCTGAAG-′3 for *ASC*; 5′-TCTGTATTCACGCCCTGTTG-′3 and 5′-TTTGCCCTCA GGATCTTGTC-′3 for *Caspase-1*; 5′-AGCCATGTACGTAGCCATCC-′3 and 3′-CTCTCAGCTGTGGTGGTGAA-′5 for β-actin as housekeeping. Data were calculated as relative expression levels using the comparative Ct method (ΔΔCt).

Hepatic-oxidized protein content assessment

Liver-oxidized protein content was measured spectrophotometrically (350–390 nm) to determine concentration of carbonyls, and at 280 nm to determine total protein concentration [22]. Values were expressed as nmol carbonyls/mg protein.

Statistical analysis

All data are presented as means ± SEM. Statistical analyses were performed with GraphPad Prism software, version 6.0 (GraphPad Software, Inc., San Diego, CA, USA). Two-way analysis of variance and Bonferroni's test were used to assess the statistical significance of differences among the groups, with $P < 0.05$ considered significant.

Results

Obesity-related parameters: Body weight, VAT, serum cholesterol, and HOMA-IR

The initial BW did not statistically differ among the groups. The HFD significantly increased BW by 57%, and the ratio of adipose tissue to BW by 321% compared with the CD. Under these conditions, HFD + RM significantly prevented an increase in BW by 31% compared with mice fed an HFD. Furthermore, the ratio of adipose tissue to BW decreased in 37% of the mice fed HFD + RM, although it did not normalize to CD values. Nevertheless, HFD + RM⁻ demonstrated a similar adipose tissue/BW ratio compared with HFD (Table 2). Serum cholesterol levels were enhanced in 30% of the HFD-fed mice compared with CD-fed animals, although no significant differences were observed among the HFD, HFD + RM, and HFD + RM⁻ groups (Table 2). Concerning HOMA-IR determination, HFD increased this index in 97% compared with CD, whereas HFD + RM decreased it in 34% compared with HFD; on the contrary, HFD + RM⁻ showed values statistically similar to HFD (Table 2). These changes were induced without significant differences in the mice food intake (data not shown), and despite the fact that the three HFD-fed groups

Table 2
General parameters in the different experimental groups: Body and abdominal adipose tissue weight, serum cholesterol, and HOMA-IR

Parameter	CD	HFD	HFD + RM	HFD + RM⁻
Initial BW, g	14.8 ± 0.5	13.8 ± 0.4	14.7 ± 0.8	14.6 ± 0.3
Final BW, g	25.3 ± 0.8^{c}	39.8 ± 1.6^{a}	27.4 ± 2.1^{c}	33.6 ± 2.9^{b}
Visceral adipose tissue, g	0.4 ± 0.03^{c}	2.3 ± 0.2^{a}	1.0 ± 0.2^{b}	1.9 ± 0.3^{a}
Adipose tissue/BW ratio ×100	1.4 ± 0.1^{c}	5.9 ± 0.3^{a}	3.7 ± 0.5^{b}	5.4 ± 0.5^{a}
Serum cholesterol, mg/dL	121 ± 12^{b}	172 ± 10^{a}	175 ± 16^{a}	187 ± 12^{a}
HOMA-IR	6.1 ± 0.2^{a}	12 ± 0.9^{b}	7.9 ± 0.5^{b}	11 ± 0.6^{b}

BW, body weight; CD, control diet; HFD, high-fat diet; HFD + RM, HFD plus Rosa mosqueta oil; HFD + RM⁻, HFD plus RM without tocopherols; HOMA-IR, homeostatic model assessment for assessing insulin resistance; RM, Rosa mosqueta oil.
Different letters above the bars indicate statistically significant differences ($P < 0.05$; two-way analysis of variance and Bonferroni test).

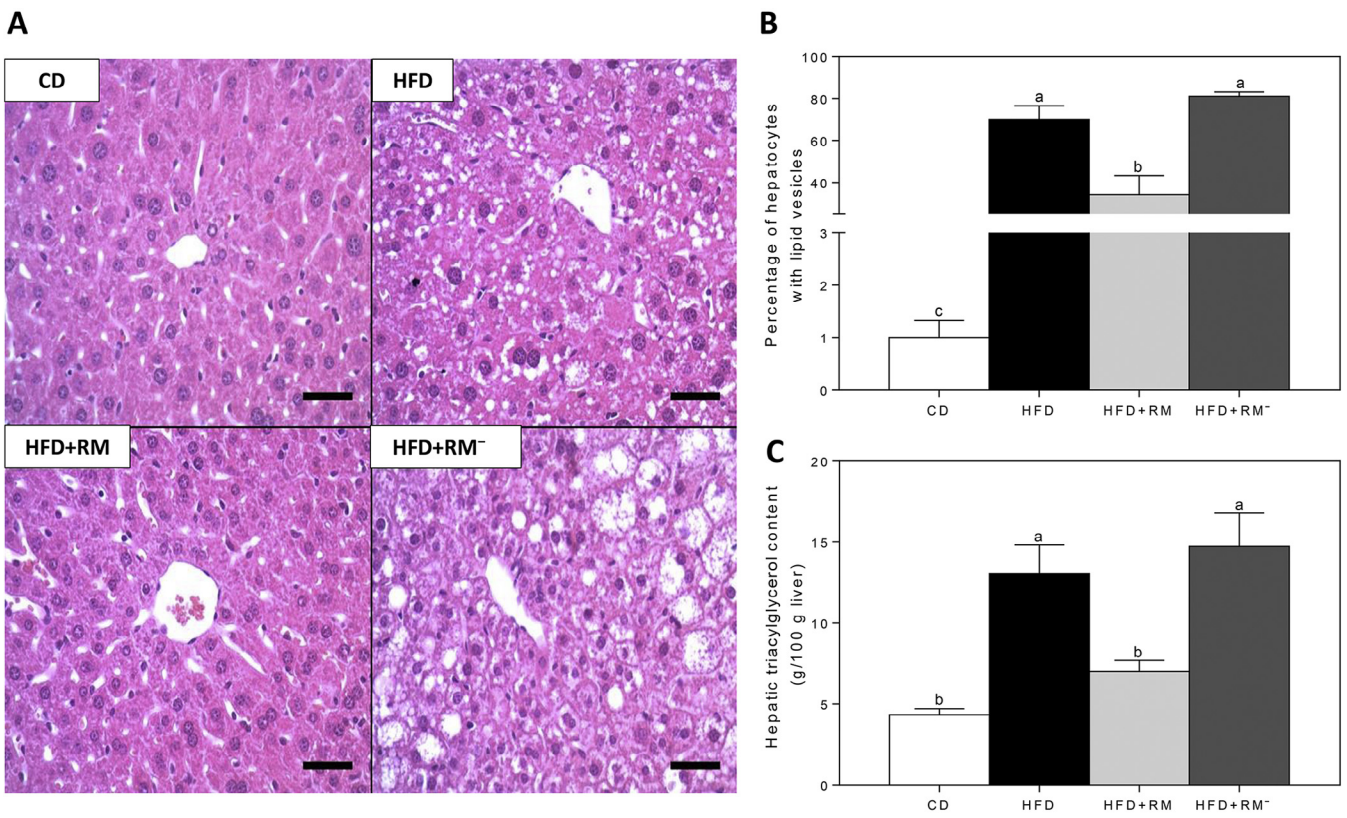

Fig. 1. Effects of α- and γ-tocopherol elimination from Rosa mosqueta oil (RM) on hepatic steatosis in in C57 BL/6 J mice subjected to a high-fat diet (HFD). (**A** and **B**) Liver histology (400×) and determination of hepatocytes with lipid vesicles, and (**C**) quantification of liver triacylglycerol content. Animals were given control diet (CD), high-fat diet (HFD), HFD plus RM (HFD + RM), or HFD plus RM without tocopherols (HFD + RM⁻). Values are expressed as mean ± SEM (n = 9). Different letters above the bars indicate statistically significant differences ($P < 0.05$; two-way analysis of variance and Bonferroni test). Scale bar = 50 μm.

consumed equivalent amounts of kcal either at the beginning or end of the treatment (Supplementary Table S2).

Parameters of hepatic steatosis

Liver histology for all groups preserved the histoarchitecture without any signs of necrosis or fibrosis. The counting for hepatocytes with lipid vesicles showed a 700% increase in micro and macro lipid vesicles in HFD-fed mice compared with the CD mice (CD, 1 ± 0.3 versus HFD, 70 ± 7), whereas HFD + RM significantly diminished lipid vesicles by 58% in relation to HFD (HFD + RM, 34 ± 9 versus HFD, 70 ± 7); nevertheless, HFD + RM$^-$ did not exhibit the same protective effect observed in HFD + RM, showing similar values as those observed in HFD-fed animals (HFD + RM$^-$, 81 ± 2 versus HFD, 70 ± 7; Fig. 1A, B).

It was determined that HFD increased hepatic TG content by 201% compared with CD (CD, 4.3 ± 0.4 versus HFD, 13 ± 2), whereas 46% of HFD + RM mice had significantly diminished TGs compared with HFD (HFD, 13 ± 2 versus HFD + RM, 7 ± 1); on the contrary, HFD + RM$^-$ did not decrease TGs as observed in HFD + RM, showing values statistically similar to the HFD (HFD + RM$^-$, 15 ± 2 versus HFD, 13 ± 2; Fig. 1C).

Adipocyte size and adipose tissue inflammation

In HFD-fed mice, an increase of 189% in adipocyte area was observed compared with CD mice (CD, 4847 ± 317 μm^2 versus HFD, 13 993 ± 1325 μm^2), whereas in HFD + RM mice the diminution was 53% compared with HFD mice (HFD, 13 993 ± 1325 μm^2 versus HFD + RM, 6590 ± 701 μm^2); additionally, no difference in adipocyte size was observed in the HFD + RM group compared with the CD mice. Nonetheless, HFD + RM$^-$ did not prevent an increase in adipocyte area as observed in HFD + RM, showing values statistically comparable to the HFD mice (HFD, 13 993 ± 1325 μm^2 versus HFD + RM$^-$ 10 929 ± 1531 μm^2; Fig. 2A, B).

HFD increased TNF-α mRNA expression by 290% compared with CD (HFD, 4 ± 1 versus CD, 1), whereas HFD + RM significantly decreased TNF-α mRNA levels compared with HFD. Furthermore, HFD + RM showed similar TNF-α values to CD (CD, 1 versus HFD + RM, 1 ± 0.1). However, HFD + RM$^-$ did not prevent the increase in TNF-α expression observed in HFD + RM, showing TNF-α mRNA values to not be statistically different from those observed in HFD-fed mice (HFD, 4 ± 1 versus HFD + RM$^-$, 6 ± 2; Fig. 2C). A HFD increased IL-1 β expression by 360% compared

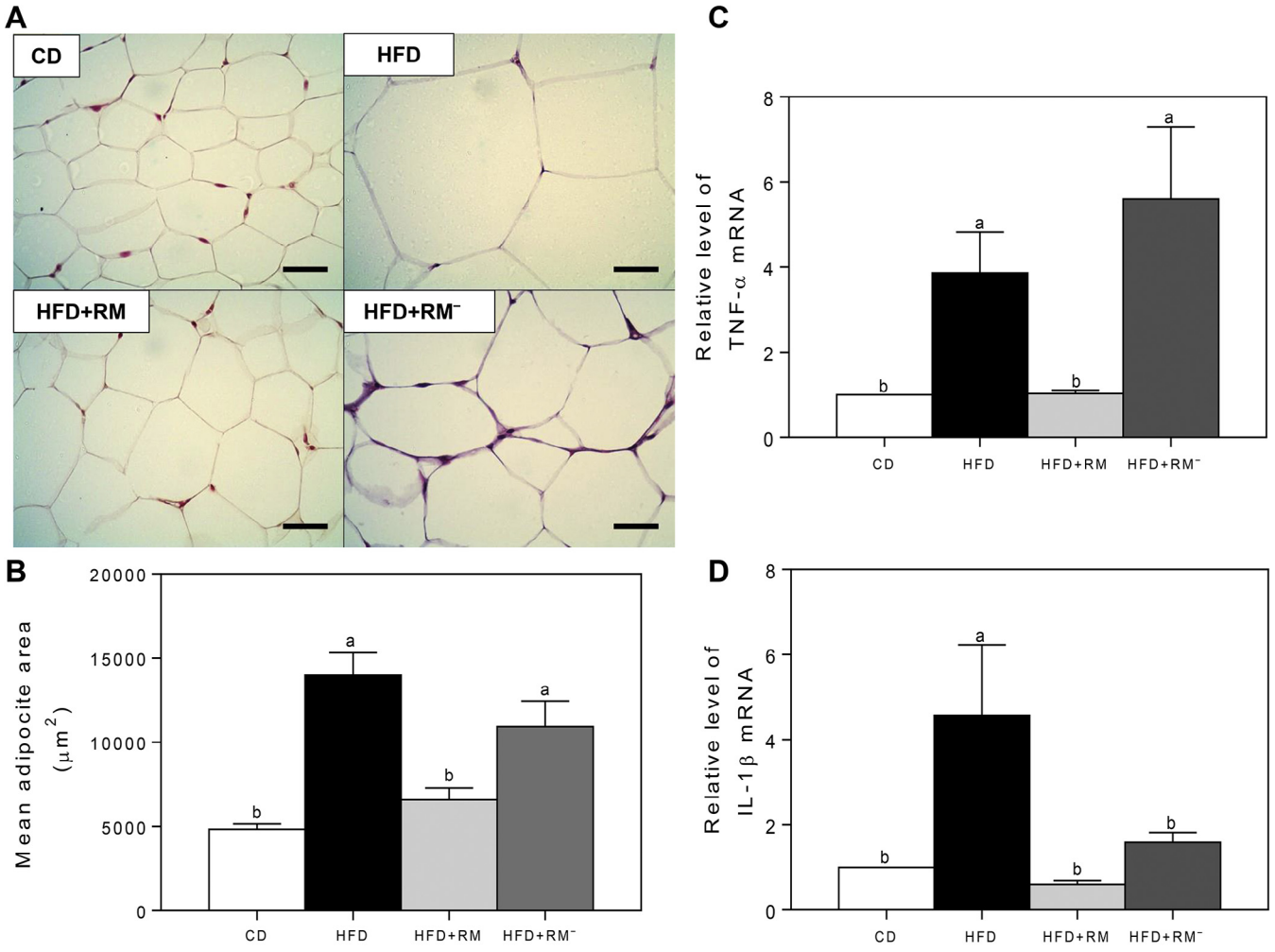

Fig. 2. Effect of α- and γ-tocopherol elimination from Rosa mosqueta oil (RM) on (**A**) visceral adipose tissue histology (400×), (**B**) adipocyte area and adipose tissue mRNA for (**C**) TNF-α and (**D**) IL-1 β in C57 BL/6 J mice subjected to a high-fat diet (HFD). Animals were given control diet (CD), HFD, HFD plus RM (HFD + RM), or HFD plus RM without tocopherols (HFD + RM$^-$). Values are expressed as mean ± SEM (n = 9). Different letters above the bars indicate statistically significant differences ($P < 0.05$; two-way analysis of variance and Bonferroni test). Scale bar = 50 μm.

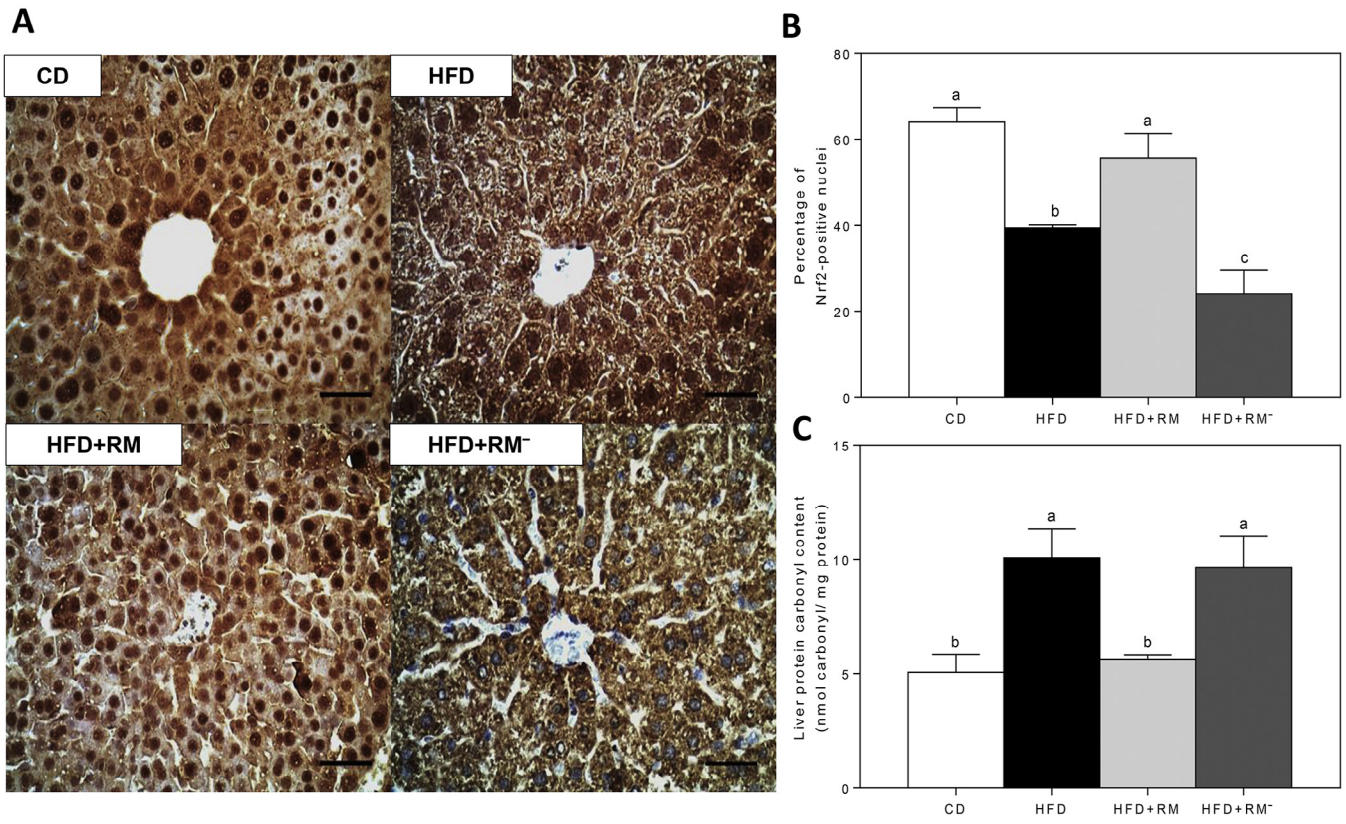

Fig. 3. Effect of α- and γ-tocopherol elimination from Rosa mosqueta oil (RM) on liver oxidative stress parameters in C57 BL/6 J mice subjected to a high-fat diet (HFD). (**A** and **B**) determination of nuclei positive for Nrf2 (400×), (**C**) liver protein carbonyl assessment. Animals were given control diet (CD), (HFD, HFD plus RM (HFD + RM), or HFD plus RM without tocopherols (HFD + RM⁻). Values are expressed as mean ± SEM (n = 9). Different letters above the bars indicate statistically significant differences ($P < 0.05$; two-way analysis of variance and Bonferroni test). Scale bar = 50 μm.

with CD (CD, 1 versus HFD, 5 ± 2), whereas HFD + RM decreased IL-1 β mRNA levels in 65% compared with HFD (HFD, 5 ± 2 versus HFD + RM, 0.6 ± 0.1); under these conditions, HFD + RM⁻ presented comparable IL-1 β mRNA levels in relation to CD and HFD + RM (CD, 1 versus HFD + RM, 0.6 ± 0.1 versus HFD + RM⁻, 1.6 ± 0.2; Fig. 2D).

Parameters of oxidative stress

Liver nuclear Nrf2 levels and oxidized protein

The HFD significantly decreased the Nrf2-positive nuclei in 67% compared with CD (CD, 65 ± 4% versus HFD, 39 ± 1%), whereas HFD + RM was effective in preventing this decrease (HFD, 39 ± 1% versus HFD + RM, 56 ± 6%), recovering the percentage of Nrf2-positive nuclei to CD values. Nonetheless, HFD + RM⁻ did not protect against HFD-induced Nrf2 depletion, showing a percentage of Nrf2-positive nuclei that was significantly lower than observed in HFD-fed mice (HFD, 39 ± 1% versus HFD + RM⁻, 24 ± 6%; Fig. 3A, B).

The HFD exhibited a 98% increased protein carbonyl content compared with CD (CD, 5 ± 1 versus HFD, 10 ± 1 nmol carbonyl/mg protein), whereas HFD + RM showed a 45% lower protein carbonyl group compared with HFD (HFD, 10 ± 1 versus HFD + RM, 5.6 ± 0.2 nmol carbonyl/mg protein). Nevertheless, HFD + RM⁻ showed similar values of carbonylated proteins to HFD (HFD, 10 ± 1 versus HFD + RM⁻, 10 ± 1 nmol carbonyl/mg protein; Fig. 3C).

Parameters of inflammation

Hepatic NF-κB activation

The HFD significantly increased the number of NF-κB–positive nuclei by 154% compared with CD (CD, 2.4 ± 0.1% versus HFD, 6 ± 1%) and, under these conditions, HFD + RM showed a depletion in the nuclear NF-κB presence by 64% compared with the HFD (HFD, 6 ± 1% versus HFD + RM, 2.2 ± 0.2%), reaching values similar to those observed in CD. On the other hand, HFD + RM⁻ showed similar nuclear NF-κB levels compared with HFD (HFD, 6 ± 1% versus HFD + RM⁻, 4 ± 1%; Fig. 4A, B).

NLRP3 inflammasome component expression in adipose tissue

The HFD increased *NLRP3* mRNA levels by 1150% compared with CD (CD, 1 versus HFD, 13 ± 4), whereas HFD + RM showed a 96% lower *NLRP3* mRNA content than HFD (HFD, 13 ± 4 versus HFD + RM, 0.5 ± 0.1), without significant differences compared with CD (CD, 1 versus HFD + RM, 0.5 ± 0.1); under these conditions, HFD + RM⁻ exhibited comparable *NLRP3* mRNA levels in relation to CD and HFD + RM (CD, 1 versus HFD + RM, 0.5 ± 0.1 versus HFD + RM⁻, 1.7 ± 0.3; Fig. 5A). Caspase-1 expression showed a similar outcome with a 990% increase in HFD compared with CD (CD, 1 versus HFD, 11 ± 3), whereas HFD + RM exhibited a significant decrease in caspase-1 mRNA levels by 65% compared with HFD (HFD, 11 ± 3 versus HFD + RM, 0.3 ± 0.1). HFD + RM⁻ presented similar caspase-1 mRNA levels compared with both CD

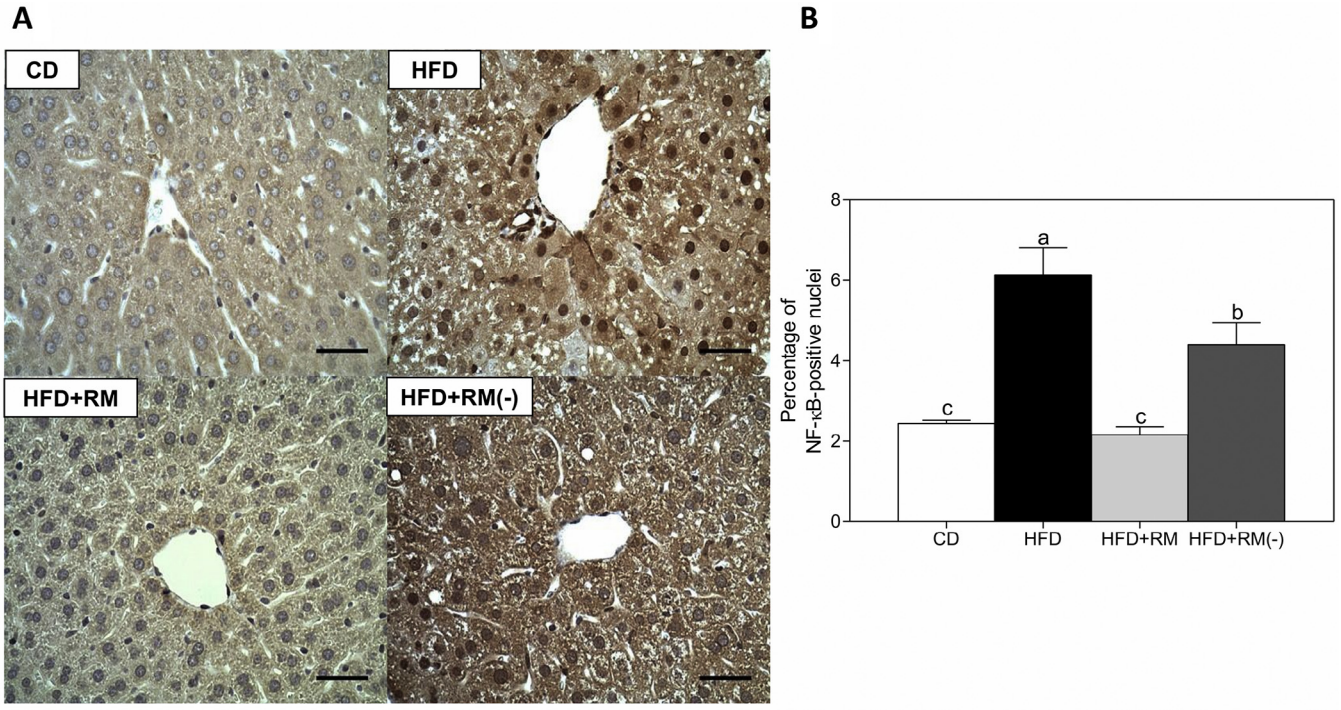

Fig. 4. Effect of α- and γ-tocopherol elimination from Rosa mosqueta oil (RM) on (**A**) and (**B**) immunohistochemical determination of positive nuclei for liver NF-κB (400×) in C57 BL/6 J mice subjected to a high-fat diet (HFD). Animals were given control diet (CD), HFD, HFD plus RM (HFD + RM), or (HFD plus RM without tocopherols (HFD + RM$^-$). Values are expressed as mean ± SEM (n = 9). Different letters above the bars indicate statistically significant differences ($P < 0.05$; two-way analysis of variance and Bonferroni test). Scale bar = 50 μm.

and HFD + RM (CD, 1 versus HFD + RM, 0.3 ± 0.1 versus HFD + RM$^-$, 1.5 ± 0.3; Fig. 5C).

It is interesting to note that no significant differences in relative ASC mRNA expression were observed among any of the experimental groups (Fig. 5B).

Discussion

Our group previously demonstrated that RM administration to mice fed a HFD prevents development of MetS, depletes prolipogenic markers, and activates Nrf2 and prolipolytic transcription factors [11–13]. In the present study, we tested the effect of α- and γ-tocopherol elimination from RM over the grade of protection against the development of MetS. We demonstrated that administration of RM$^-$ to HFD-fed mice did not prevent the following events: liver steatosis (Fig. 1), adipocyte hypertrophy (Fig. 2), oxidative stress (Fig. 3), and proinflammatory state (Figs. 3–5). RM contains levels of α- and γ-tocopherol in a 1:5 ratio (Table 1), and the experimental results obtained from the present investigation reinforced the importance of tocopherols in the RM-dependent amelioration of chronic alterations induced by nutritional factors, which could be explained by antioxidant properties such as reactive oxygen and nitrogen species scavengers as well as the indirect non-antioxidant biological activity of some endogenous, highly bioactive long-chain metabolites (LCMs) derived from vitamin E by ω-hydroxylation or ω-oxidation, such as 13′-hydroxychromanol, 13′-carboxychromanol, γ-12′-hydroxychromanol, and γ-11′-hydroxychromanol [23,24].

A growing body of evidence supports the healthy properties of tocopherols, principally their peroxyl radical-trapping antioxidant activity, which finishes the propagation of lipoperoxidation. It is well documented that α-tocopherol is the major form of vitamin E with the highest affinity of hepatic α-tocopherol transfer protein, higher tissue content, and greater antioxidant capacity than other vitamin E forms [23], but γ-tocopherol is superior to α-tocopherol in detoxifying electrophile compounds such as nitrogen dioxide and peroxynitrite by the formation of 5-nitro-γ-tocopherol [19,23]. In biological systems, it has been demonstrated that γ-tocopherol decreases ex vivo superoxide anion (O_2^-) generation, lipoperoxidation, and low-density lipoprotein (LDL) oxidation in thrombotic rats [25]. Also, it has been shown that oxidative stress–induced IR was improved by pretreatment with γ-tocopherol in rat L6 skeletal muscle cells [26]. It also has been found that administration of argan oil (rich in α- and γ-tocopherols) to rats who were fed glucose for 5 wk prevents arterial hypertension, hyperglycemia, IR, and decreases O_2^- production, and NADPH oxidase activity [27]. On the other hand, Nrf2 activation by RM can be explained by direct tocopherol functions, of which a number of studies have demonstrated that α- and γ-tocopherols likely promote Nrf2 activity by producing a small amount of oxidative stress, inducing cytoprotection and cell survival [28–30], although it also has been postulated that antioxidant and antiinflammatory effects of tocopherols are independent of Nrf2 activity [31]. The above-mentioned antioxidant α- and γ-tocopherol effects could have a direct effect on the prevention of hepatic steatosis by RM; in this sense, it has been demonstrated that both α- and γ-tocopherols decreased oxidative stress, inflammation, and TG accumulation into hepatocytes in obese mice with LPS-induced steatohepatitis and in steatohepatitic patients [19,32], which underlines the importance of treating MetS markers by an antioxidant approach. On the other hand, it has been reported that α-tocopherol prevents LDL-stimulated vascular smooth muscle cell proliferation by modulating protein kinase C activity

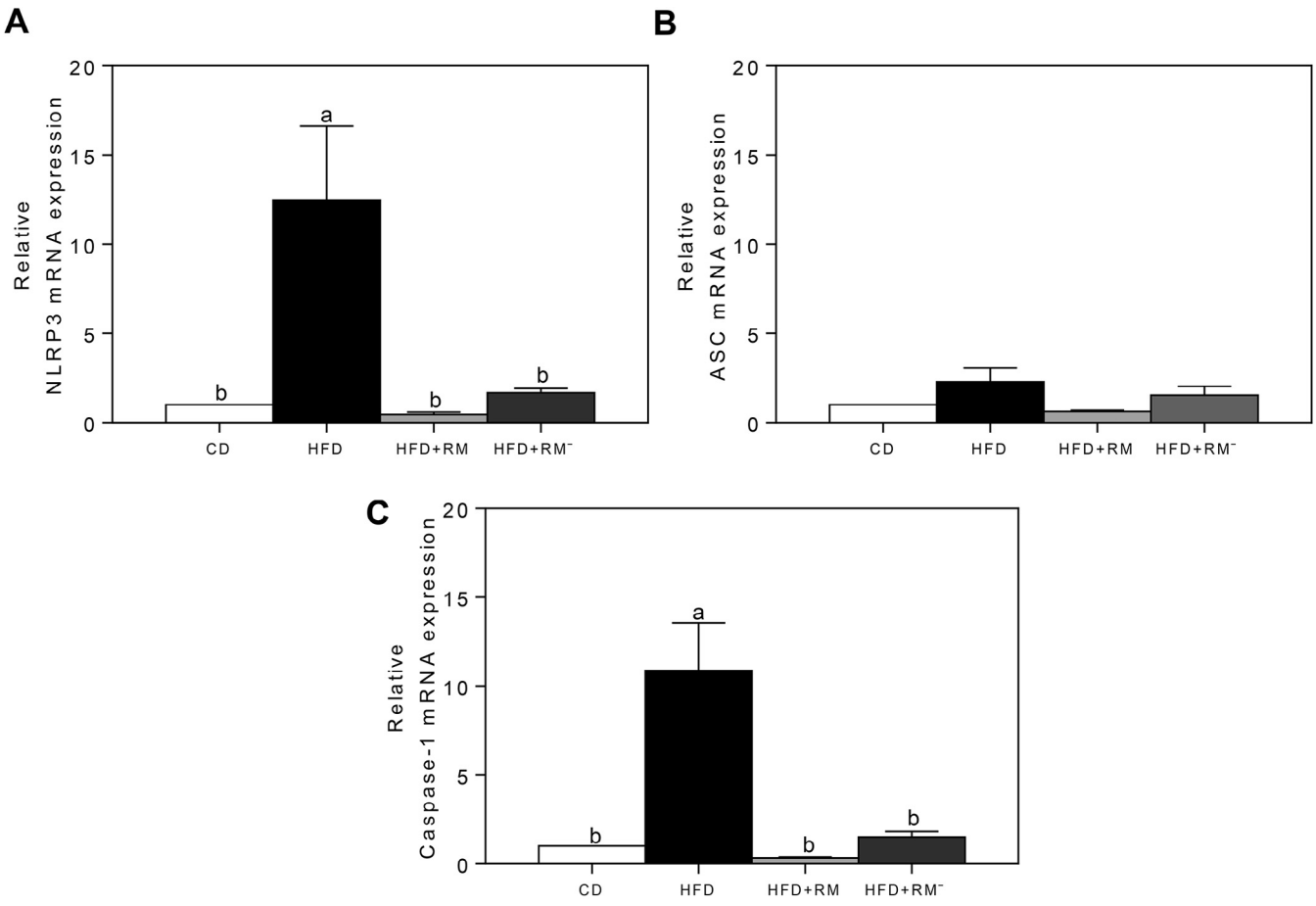

Fig. 5. Effect of α- and γ-tocopherol elimination from Rosa mosqueta oil (RM) on NLRP3 inflammasome expression in C57 BL/6 J mice subjected to a high-fat diet (HFD). Adipose tissue mRNA for (**A**) *NLRP3*, (**B**) apoptosis-associated Speck-like protein (*ASC*), and (**C**) *caspase-1*. Animals were given control diet (CD), HFD, HFD plus RM (HFD + RM), or (HFD plus RM without tocopherols (HFD + RM⁻). Values are expressed as mean ± SEM (n = 9). Different letters above the bars indicate statistically significant differences ($P < 0.05$; two-way analysis of variance and Bonferroni test).

in vitro [33,34], and vitamin E administration decreases c-Jun N-terminal kinase phosphorylation and inhibits matrix metallopeptidase 9 expression, thus preventing inflammation and monocytes infiltration [35]. Non-antioxidant LCM activity plays an important role in vitamin E–dependent healthy properties, considering that LCM can modulate signaling cascades and/or regulates gene expression; for instance, in vitro administration of α-LCM to human macrophages decreased uptake of oxidized LDL (oxLDL) and oxLDL-induced lipid accumulation, as well as induces expression of the scavenger receptor CD36 in macrophages and liver, thus reducing the risk for atherosclerosis and cardiovascular diseases [24].

Both, α- and γ-tocopherol have strong antiinflammatory properties, not only due to their direct antioxidant activities but also because of the modulation of molecular targets linked to inflammatory processes [36], even demonstrating that γ-tocopherol has greater antiinflammatory properties than α-tocopherol [28]. For instance, it has been reported that γ-tocopherol decreases production of arachidonic acid (AA)–derived prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) by downregulation of cyclooxygenase (COX)-2 and 5-lipoxygenase (5-LOX) [37]. Furthermore, inhibition of the Akt phospholyration by α-tocopherol has antiinflammatory actions either by inhibiting proliferation of inflammatory cells and/or inhibition of ERK1/2, p38 MAPK and Akt-mediated NF-κB DNA-binding activity [38,39], which is important as NF-κB is a master regulator that enhances expression of TNF-α, IL-6, IL-1 β, and another inflammatory cytokines and adhesion molecules by inflammatory stimuli [36]. Furthermore, α-LCMs can also exert their antiinflammatory properties by blocking the LPS-induced expression of nitric oxide synthase, COX-2, and IL-1 β [24]. All the above-mentioned antiinflammatory effects of α- and γ-tocopherol would explain the results showing that dietary RM⁻ did not prevent excess of fat mass nor the adipocyte hypertrophy, as observed in HFD + RM (Table 2; Fig. 2). Surprisingly, results from the present study demonstrated that RM prevented HFD-induced expression of NLRP3 inflammasome regardless of the presence or absence of tocopherols (Fig. 5). In response to palmitate, ceramides, and other damage-associated molecular patterns [40], NLRP3 inflammasome activates processing and secretion of IL-1 β, cytokine associated with IR, T2DM, and MetS [41]. Although oxidative stress is one of the factors that modulates NLRP3 inflammasome assembly [42], we speculate that hepatic ALA bioconversion to eicosapentaenoic acid (EPA)/DHA and their accumulation in the VAT [11,43] would be enough to give an account for RM-dependent decreased expression of NLRP3 inflammasome components, thus ALA bioconversion would contribute anyway to the prevention of the proinflammatory state development as EPA and DHA can block NLRP3 inflammasome activation, decreasing IR and increasing insulin sensitivity [44].

Conclusion

These data make it possible to establish that tocopherols from RM play an important role in the prevention of hepatic steatosis, adipocyte enlargement, oxidative stress, and inflammation induced by a HFD, and supports the potential use of tocopherol-rich vegetal sources in the treatment against MetS.

Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.nut.2018.01.012.

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