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ORIGINAL CONTRIBUTION

Effect of consumption of tomato juice enriched with n-3 polyunsaturated fatty acids on the lipid profile, antioxidant biomarker status, and cardiovascular disease risk in healthy women

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Abstract
Purpose We compared the effects of consumption of n-3 polyunsaturated fatty acids (PUFA)-enriched tomato juice versus plain tomato juice on the serum lipid profile and levels of biomarkers related to antioxidant status and cardiovascular disease (CVD) risk in women.
Methods Eighteen healthy women participated in a 2-week intervention trial involving the daily intake of 500 mL of n-3 PUFA-enriched juice ($n = 11$) or plain tomato juice ($n = 7$). Each serving of enriched juice provided 250 mg of eicosapentaenoic acid (EPA) plus docosahexanoic acid (DHA). Both juices provided natural antioxidant compounds such as phenolics (181 mg) and lycopene (26.5 mg).
Results Intervention with the enriched juice had no effect on the lipid profile, and serum levels of triglycerides and cholesterol (total, LDL, and HDL) remained unchanged. The serum antioxidant status improved following juice intake, as revealed by an increase in total antioxidant capacity and a slight decrease in lipid peroxidation. The serum levels of homocysteine, a cardiovascular risk factor, decreased following n-3 PUFA-enriched juice consumption. A decrease in vascular adhesion molecule 1 (VCAM-1) levels was also noted after intake of either plain or enriched tomato juice, whereas intercellular adhesion molecule 1 (ICAM-1) levels only decreased following intake of the enriched juice.
Conclusions Overall, stronger positive amelioration of CVD risk factors was observed following the intake of n-3 PUFA-enriched juice than after plain tomato juice consumption, which suggested a possible synergistic action between n-3 PUFAs and tomato antioxidants.

Keywords Cardiovascular diseases (CVD) · PUFAs · Tomato juice · Antioxidants · Homocysteine · Lipid oxidation · VCAM-1 · ICAM-1

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Introduction

Cardiovascular diseases (CVDs) are responsible for significant mortality and morbidity throughout the world [1] and also create high healthcare costs in the EU and USA [2, 3]. A number of risk factors are associated with CVD, including high cholesterol levels, low HDL cholesterol, high homocysteine levels, obesity, diabetes, hypertension, and low levels of antioxidants [4]; however, many of these risk factors can be modulated by a healthy balanced diet and lifestyle. In this regard, a substantial body of evidence indicates that n-3 polyunsaturated fatty acids (n-3 PUFAs) play an important role in CVD prevention by increasing HDL cholesterol, endothelium-dependent vasodilation, atherosclerotic plaque stability, and production of vasoconstrictive eicosanoids [5–10]. The n-3 PUFAs also have been reported to decrease triglyceride levels, platelet aggregation and adhesion, adiponectin levels, blood pressure, and heart rate, as well as levels of inflammation markers such as IL-6 and TNF-α [1, 9, 11, 12]. Research

dealing with n-3 PUFAs has generally focused on eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA), which are endogenously derived from the essential fatty acid, α-linolenic acid (ALA), by a process involving elongation and desaturation. However, the general belief is that the rates of endogenous EPA and DHA production are small. Approximately, 5–10% of dietary ALA can be converted to EPA, and an additional 2–5% may be further converted to DHA in healthy adults [1, 8, 13].

For these reasons, daily diets should ensure adequate intake of n-3 PUFAs. The major source of EPA and DHA in the diet is oily fish such as tuna, salmon, cod, swordfish, trout, and mackerel [9], while ALA is found in dairy products, organ meats such as liver, vegetable oils like soybean, linseed, and canola, as well as in green leafy vegetables [1, 13]. Recommended intakes for n-3 PUFAs are not uniform. Various organizations worldwide have made dietary recommendations for EPA+DHA and fish intake in order to reduce the risk of CVD. For instance, the World Health Organization (WHO) recommends regular fish consumption—one to two servings per week—in order to provide around 200–500 mg EPA+DHA/per serving [14]. Similarly, other organizations recommend weekly fish intake in order to provide EPA+DHA in amounts within the range of 400–670 mg/day [15, 16]. Nevertheless, current intakes of EPA and DHA are still low in most individuals living in Western countries [8].

In this context, incorporation of microencapsulated fish oil into food products provides a means of increasing n-3 PUFA intake, particularly in populations where fish intake remains low [17]. A wide range of food products have been enriched with n-3 PUFAs, including dairy products (milk, yogurt, cheese, and butter), breakfast cereals, bread, eggs, meat, fruit juice, nutrition bars, salad dressing, margarines, infant formula, and baby foods [18]. An important issue to be taken into consideration is the food product used for enrichment, as the food matrix can influence n-3 PUFA availability and consumer acceptance [17, 19–21]. From both a nutritional and technological point of view, tomato juice could be a suitable matrix to serve as a vehicle for n-3 PUFAs, and the designing of functional foods to prevent CVD disease could be interesting, given the beneficial properties of both fish oil and tomato consumption.

Raw tomatoes and tomato juice provide an optimal mix of dietary phytochemicals such as carotenoids, phenolic compounds, and antioxidant vitamins C and E, and these remain more or less stable throughout the shelf life [22, 23]. Tomato product consumption in itself has been associated with a lower CVD risk, an effect generally attributed to antioxidant content, especially lycopene [24, 25]. Tomatoes also contain folates [24, 26], which play an important role in the lowering of homocysteine levels and therefore CVD risk [27]. The beneficial effects of tomato juice are a result of the interactions among different compounds and could be increased by the addition of other functional ingredients, which could have a synergistic effect with the existing natural components. For example, the consumption of tomato juice enriched with ascorbic acid reduces the levels of some biomarkers of oxidative stress and inflammation that are related to CVD disease [28], due to the synergistic action of tomato bioactive compounds and the added vitamin C.

According to this criterion, the addition of fish oil with n-3 PUFAs to tomato products could possibly result in a synergistic action between n-3 PUFAs and bioactive tomato compounds in exerting beneficial effects against CVD. The objective of the present study was to investigate the effects of consumption of n-3 PUFA-enriched tomato juice, versus plain tomato juice, on the serum lipid profile and on levels of biomarkers related to antioxidant status and CVD risk in healthy adult women.

Materials and methods

Test product

Plain tomato juice and the same tomato juice enriched with n-3 PUFAs were provided by a local foodstuff industry, Hero España, S.A. (Alcantarilla, Murcia) for use in the intervention study. Variations in the nutrient composition were avoided by ensuring that all of the juices used in the study belonged to the same batch. The tomato juice was enriched with microencapsulated n-3 PUFA powder from cod liver oil (Denomega™ Powder DS) provided by Denomega Nutritional Oils (Sarpsborg, Norway). The nutrient and bioactive compound composition of the tomato juices used in this study are shown in Table 1.

Subjects

A total of 22 women were recruited from a pool of volunteers from the staff of the Santa María del Rosell Hospital (Cartagena, Spain). The subjects were aged 35–55 years, with a body mass index ranging from 21 to 30 kg/m^2. They were non-smokers and did not take any medication, vitamin/mineral supplements, or oral contraceptives. All participants were in good health, based on their medical history, a medical examination, and normal results from clinical laboratory tests. The protocol was carefully explained to the volunteers, and their written informed consent was obtained. The study was approved by the Clinic Research Ethics Committee of the Santa María del Rosell Hospital and complied with the Helsinki guidelines for clinical studies.

Table 1 Nutrient and bioactive compound composition of the tomato juices used in the study expressed per 100 mL (data into parenthesis represent the content per 500 mL of tomato juice)

Compound	Amount per 100 mL of juice	
	Reference juice	n-3 PUFA-enriched juice
Energy (kcal)	17 (85)	20.4 (102)
Protein (g)	0.8 (4)	0.8 (4)
Total carbohydrates (g)	3.5 (17.5)	3.5 (17.5)
Total fat (g)[a]	0.1 (0.5)	0.36 (1.8)
Total n-3 PUFAs (mg)	–	65 (325)
EPA (mg)	–	25 (125)
DHA (mg)	–	25 (125)
Other n-3 PUFAs (mg)	–	15 (75)
Bioactive compounds		
Total phenolics (mg)	36.2 (181)	36.2 (181)
Lycopene (mg)	5.3 (26.5)	5.3 (26.5)
Folates (μg)	5.2 (26)	5.2 (26)
Minerals		
Iron (mg)	0.2 (1)	0.2 (1.0)
Zinc (mg)	0.1 (0.5)	0.1 (0.5)

[a] Total fat amount includes the sum of saturated, monounsaturated and polyunsaturated fatty acids added with the Denomega™ Powder DS mono-dose bag

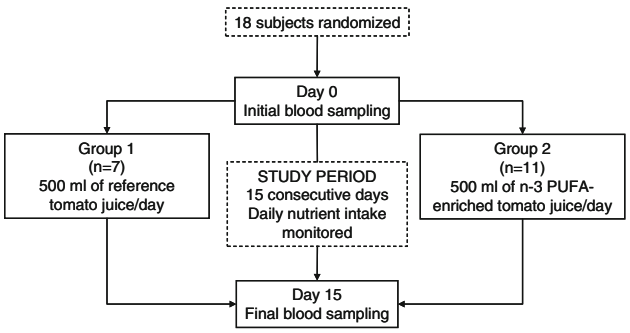

Fig. 1 Design of the intervention study

Study design

The present study was designed as a randomized single-blind intervention trial with a period of 2 weeks (Fig. 1). In brief, subjects were randomly divided into two groups (11 reference group and 11 test group) who were informed to intake 500 mL of either the reference tomato juice or n-3 PUFA-enriched tomato juice every day during the 2-week study period. However, on the initial day, two subjects withdrew from the study because they started taking medication, and another two subjects left the trial due to dislike of the test product. These four subjects belonged to the reference juice group. The juices were ingested in this way: 250 mL in the morning and 250 mL in the late afternoon, but they never replaced a meal. Subjects were also instructed to go on with their habitual lifestyle, physical activity, and dietary habits. Their habitual diets were checked daily with 24 h dietary recalls and were evaluated with the Program "Alimentación y Salud" (Versión 0698.046, BitASDE general Médica farmaceútica, Valencia, Spain). At days 0 and 15, blood samples were collected in BD Vacutainer™ SSTTMII Advance tubes (Becton–Dickinson, Madrid, Spain) to perform the different analyses. Serum was obtained by centrifugation at 5,000 rpm for 5 min at 4 °C, and serum samples were stored at −80 °C until analyzed. Body mass indexes were calculated at the beginning and at the end of the study to eliminate the participation of obese women. Systolic and diastolic blood pressures were also measured on the left arm during the intervention study, and all showed values within normal limits.

Blood analyses

Baseline data collection included complete blood count and blood analyses. Some of these parameters are not reported in the "Results" section, because they did not show significant differences between groups at the beginning or at the end of the study. Other parameters regarding the aims of the study are referenced below. The total cholesterol content was measured using the CHOD-PAP Cholesterol kit (#1.489.232). The total LDL and total HDL cholesterol were determined using Roche kits (#0-674) and (#04714423), respectively, whereas serum triglycerides (TGA) were measured using the Triglycerides GPO-PAP kit (#1.488.872). All readings were taken in a Cobas Integra 400 automated analyzer (Roche Diagnostics, Mannheim, Germany). Atherogenicity Index or Castelli Risk Index was calculated according to Castelli [29]. The level of serum lipid peroxidation was evaluated as the concentration of serum thiobarbituric acid reactive substances (TBARS) following the methodology previously described [30, 31]. The serum total antioxidant capacity was evaluated by the Trolox equivalent antioxidant capacity assay (TEAC assay) [32] and the ferric reducing/antioxidant power (FRAP assay) [33]. For both tests, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) (Sigma, St. Louis, USA) was used for calibration. Serum albumin, uric acid, and bilirubin concentrations were measured in a Roche/Hitachi 904 automated clinical chemistry analyzer using commercially available Roche kits ALB plus (#1.970.569), UA plus (#1.661.850), and BIL-T (#1.489.194), respectively. Serum ascorbic acid was analyzed by DAD reverse-phase HPLC (Merck-Hitachi L-2200, Darmstadt, Germany) [34]. Serum β-carotene was determined by DAD reversed-phase HPLC (Merck-Hitachi L-2200, Darmstadt, Germany) using a Spherisorb S5 ODS1 column [35].

Serum folates were analyzed using the Elecsys Folate 2 kit (#03253678122, Roche Diagnostics, Mannheim, Germany). The serum vitamin B_{12} levels were analyzed by electrochemiluminescence immunoassay using the Roche Vit B_{12} kit (#1.820.753). Serum total homocysteine was analyzed by competitive fluorescence polarization immunoassay (FPIA) in an IMx automated analyzer, using the Homocysteine kit (#7D29-20) from Abbot Diagnostic Division (Dundee, UK). The C-reactive protein (CRP) concentration was determined by an immunoturbidimetric test (Tina-quant CRPLX, Roche Diagnostics, D-68298 Mannheim, Germany) using a Roche/Hitachi 904 automated clinical chemistry analyzer (Roche Diagnostics, Mannheim, Germany). The soluble forms of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) were analyzed by enzyme-linked immunosorbent assay (ELISA) using the commercially available sVCAM-1-ELISA-Kit (#KHT0611) and sICAM-1-ELISA-Kit (#KHS5411), respectively. Kits were supplied by Invitrogen S.A. (Barcelona, Spain). Readings were performed using a Power Wave XS plate reader (Biotek Instruments INC, Winooski, Vermont, USA).

Statistics

Data were analyzed using a statistical software package (SPSS 15.0; SPSS Inc., Chicago, IL, USA). Unless otherwise stated, results are presented as mean ± SEM. A Student's *t* test was performed within each study group to evaluate differences between variables at days 0 and 15. In addition, an analysis of variance (ANOVA) was also conducted between groups to determine the statistical differences between groups at day 0 and day 15. p values <0.05 were considered statistically significant.

Results

Table 1 shows the nutrient and bioactive compound composition of the tomato juices used in the study. Both juices provided antioxidant compounds such as phenolics and lycopene, and a slight folate intake. In addition, n-3 PUFA-enriched juice provided 250 mg EPA+DHA per 500 mL serving, which was equivalent to 50% of the n-3 PUFA recommended by ISSFAL, established at 500 mg/day [16]. The estimated daily intakes of macronutrients, vitamins, and minerals in both groups showed no statistically significant differences (Table 2). In general, the diets followed by the volunteers showed a similar intake of macro- and micronutrients. The intake of macronutrients was higher in protein and fat, and lower in carbohydrates, compared with the daily recommended intakes (DRIs). However, these patterns have been described in general in the diet of developed counties. The micronutrient intake fulfilled the DRI for this group, with the exception of vitamins A and D, and minerals Fe, I, and Zn.

Table 3 shows the blood parameters, blood pressure, and body mass index (BMI) measured at day 0 and at the

Table 2 Macronutrients and micronutrients intake in both group during the intervention study

Parameters	RDIs[a]	Reference juice ($n = 7$)	n-3 PUFA-enriched juice ($n = 11$)
Protein (g)	41 (10–15% TE[b])	81.2 ± 11.73	73.76 ± 16.36
Carbohydrates (g)	55–75% TE[b]	157.85 ± 32.06	148.71 ± 34.42
Dietary fiber (g)	25	18.81 ± 7.68	18.59 ± 10.2
Total fat (g)	15–30% TE[b]	55.38 ± 15.04	55.11 ± 16.95
Vitamin A (μg)	800	592.78 ± 143.88	493.16 ± 122.59
Vitamin C (mg)	60	182.85 ± 42.72	149.83 ± 32.13
Vitamin D (μg)	5	2.09 ± 0.34	2.42 ± 3.52
Vitamin E (mg)	12	11.89 ± 1.75	12.03 ± 13.88
Vitamin B_{12} (μg)	2	12.04 ± 2.41	9.13 ± 5.82
Folic (μg)	200	247.76 ± 60.77	204.53 ± 50.73
Vitamin B_6 (mg)	1.6	2.18 ± 0.41	1.87 ± 0.34
Vitamin B_2 (mg)	1.4	1.95 ± 0.62	1.57 ± 0.54
Sodium (mg)	5,000	3562.87 ± 517.08	3064.68 ± 504.73
Potassium (mg)	2,000	3502.01 ± 457.75	3014.92 ± 465.2
Calcium (mg)	800	942.14 ± 228.04	768.31 ± 206.98
Phosphorus (mg)	800	1149.31 ± 282.08	859.62 ± 263.97
Magnesium (mg)	280	280.97 ± 42.19	274.75 ± 61.12
Iron (mg)	15	13.14 ± 3.42	14.87 ± 5.33
Iodine (μg)	150	89.9 ± 9.79	61.36 ± 15.13
Zinc (mg)	12	8.91 ± 1.46	7.05 ± 1.66

Mean ± standard deviation of the daily dietary recalls during the intervention period

[a] Recommended dietary intakes for Spanish women between 19 and 50 years (Program: Alimentación y Salud. Versión 0698.046)

[b] *TE* Total energy

Table 3 Changes in blood parameters, blood pressure, and body mass index (BMI) after the intervention study

Blood parameters	Reference juice ($n = 7$)		n-3 PUFA-enriched juice ($n = 11$)		ANOVA significance		Reference
	Day 0	Day 15th	Day 0	Day 15th	Day 0	Day 15th	values
RBC $\times 10^{12}$/L	4.13 ± 0.12	4.10 ± 0.14	4.37 ± 0.07	4.35 ± 0.09	$p > 0.05$	$p > 0.05$	4–5.5 $\times 10^{12}$
Hemoglobin	12.98 ± 0.50	12.73 ± 0.45	12.90 ± 0.34	12.87 ± 0.35	$p > 0.05$	$p > 0.05$	12–16
Hematocrit	38.17 ± 1.25	37.71 ± 1.36	37.91 ± 0.81	38.00 ± 1.06	$p > 0.05$	$p > 0.05$	36–46
WBC $\times 10^{9}$/L	4.80 ± 0.42	5.62 ± 0.57	5.66 ± 0.48	5.19 ± 0.34	$p > 0.05$	$p > 0.05$	4–10 $\times 10^{9}$
Platelet $\times 10^{9}$/L	239.67 ± 17.27	259.57 ± 18.34	249.45 ± 15.17	247.72 ± 15.93	$p > 0.05$	$p > 0.05$	150–450 $\times 10^{9}$
Systolic Pres. (mmHg)	97.14 ± 3.06	105.86 ± 3.39	105.18 ± 2.63	106.36 ± 2.79	$p > 0.05$	$p > 0.05$	120
Diastolic Pres. (mmHg)	63.57 ± 2.37	65.71 ± 3.85	65.45 ± 2.82	64.09 ± 3.36	$p > 0.05$	$p > 0.05$	80
BMI	23.43 ± 1.27	23.43 ± 1.28	25.17 ± 3.07	25.18 ± 3.03	$p > 0.05$	$p > 0.05$	20–25

Table 4 Changes in serum antioxidants and parameters related to antioxidant status

Blood parameters	Reference juice ($n = 7$)		n-3 PUFA-enriched juice ($n = 11$)		ANOVA significance	
	Day 0	Day 15th	Day 0	Day 15th	Day 0	Day 15th
Total cholesterol (mg/dl)	201.86 ± 12.45	202.29 ± 8.98	192.64 ± 10.10	202.27 ± 10.28	$p > 0.05$	$p > 0.05$
HDL cholesterol (mg/dl)	76.00 ± 5.36	79.43 ± 5.51	68.91 ± 4.61	73.73 ± 4.99	$p > 0.05$	$p > 0.05$
LDL cholesterol (mg/dl)	115.21 ± 11.39	112.29 ± 10.36	110.55 ± 8.60	115.36 ± 5.45	$p > 0.05$	$p > 0.05$
Triglycerides (mg/dl)	53.00 ± 4.68	52.86 ± 3.88	65.91 ± 8.51	65.45 ± 7.93	$p > 0.05$	$p > 0.05$
Atherogenicity index	2.73 ± 0.25	2.65 ± 0.27	2.87 ± 0.19	2.81 ± 0.18	$p > 0.05$	$p > 0.05$
Albumin (g/dl)	4.49 ± 0.07	4.47 ± 0.12	4.53 ± 0.08	4.54 ± 0.06	$p > 0.05$	$p > 0.05$
Bilirubin (mg/dl)	0.67 ± 0.28	0.69 ± 0.14	0.49 ± 0.03	0.55 ± 0.05	$p > 0.05$	$p > 0.05$
Uric acid (mg/dl)	3.65 ± 0.42	3.64 ± 0.43	3.79 ± 0.23	3.68 ± 0.22	$p > 0.05$	$p > 0.05$
Ascorbic acid (mg/dl)	0.22 ± 0.04	0.27 ± 0.05	0.53 ± 0.11	0.39 ± 0.09	$p > 0.05$	$p > 0.05$
β-Carotene (mg/dl)	51.38 ± 8.87	65.87 ± 10.36	42.74 ± 6.40	45.60 ± 6.36	$p > 0.05$	$p > 0.05$
TEAC (mM Trolox)	2.17 ± 0.27	2.90 ± 0.23	2.50 ± 0.19	2.41 ± 0.25	$p > 0.05$	$p > 0.05$
FRAP (mM Trolox)	0.22 ± 0.02	0.24 ± 0.10	0.22 ± 0.01	0.27 ± 0.01***	$p > 0.05$	$p < 0.05$[a]
MDA (μM MDA)	0.62 ± 0.06	0.54 ± 0.06	0.75 ± 0.11	0.61 ± 0.03	$p > 0.05$	$p > 0.05$

[a] Significant differences in the FRAP values at day 15th between the intervention groups

*** Significantly different from day 0 within each treatment group ($p < 0.001$; Student's t test)

end of the intervention study (day 15) in both groups. No changes were observed in the studied parameters during the intervention period within each group. All parameters showed figures within the range of the reference values.

As shown in Table 4, intake of either reference or n-3 PUFA-enriched juice had no effect on serum lipid concentrations or on atherogenicity index. Similarly, no significant changes were observed during the study in serum antioxidant levels and in parameters related to antioxidant status, except for serum FRAP values that significantly increased after the intake of the n-3 PUFA-enriched juice. The changes in parameters related to cardiovascular disease risk, such as the serum levels of folates, vitamin $B_{12,}$ or CRP (Table 5), were not significantly affected by consumption of either reference or n-3 PUFA-enriched juice. However, juice intake was effective in reducing the serum concentrations of homocysteine, and the soluble forms of the adhesion molecules VCAM-1 and ICAM-1. As shown in Table 4, homocysteine levels significantly ($p < 0.01$) decreased in the group consuming the n-3 PUFA-enriched juice. Similarly, n-3 PUFA supplementation significantly ($p < 0.05$) decreased the serum levels of VCAM-1 and ICAM-1. Interestingly, reference juice intake also had a significant ($p < 0.05$) effect on reducing VCAM-1 levels during the trial.

In general, ANOVA showed no significant differences for the different studied parameters between either group at day 0 and 15. Only the homocysteine level at day 0, and FRAP and ICAM-1 values at the end of the study showed a significant difference of $p < 0.05$. These results suggest

Table 5 Changes in parameters related to cardiovascular disease risk

Blood parameters	Reference juice ($n = 7$)		n-3 PUFA-enriched juice ($n = 11$)		ANOVA significance	
	Day 0	Day 15th	Day 0	Day 15th	Day 0	Day 15th
Folates (ng/mL)	10.08 ± 0.98	10.05 ± 1.28	7.28 ± 0.53	7.24 ± 0.42	$p > 0.05$	$p > 0.05$
Vitamin B_{12} (pg/mL)	653.23 ± 129.84	708.12 ± 176.23	556.08 ± 41.55	539.88 ± 54.50	$p > 0.05$	$p < 0.05$
Homocysteine (μM)	7.40 ± 0.34	7.71 ± 0.42	10.86 ± 0.61[a]	8.42 ± 0.42**	$p < 0.05$[a]	$p > 0.05$
CRP (mg/dl)	0.10 ± 0.04	0.30 ± 0.27	0.20 ± 0.05	0.21 ± 0.05	$p > 0.05$	$p > 0.05$
VCAM-1 (μg/L)	892.02 ± 132.46	516.15 ± 73.52*	1036.91 ± 174.01	543.25 ± 57.62**	$p > 0.05$	$p > 0.05$
ICAM-1 (μg/L)	778.97 ± 61.58	702.40 ± 59.75	686.88 ± 45.43	486.93 ± 46.76**	$p > 0.05$	$p < 0.05$[b]

[a] Significant differences in the homocysteine level at day 0 between the intervention groups

[b] Significant differences in ICAM-1 level at day 15th between the intervention groups

* Significantly different from day 0 within each treatment group (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; Student's t test)

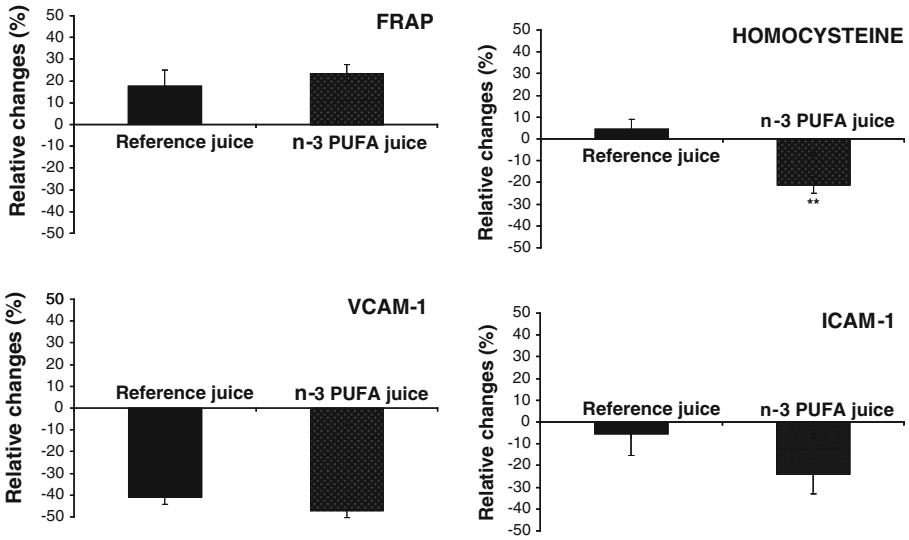

Fig. 2 Relative percent changes in serum FRAP, homocysteine, VCAM-1 and ICAM-1 concentrations in the study groups after tomato juice consumption. **Significantly different compared with reference juice group (**$p < 0.01$; Student's t test)

that volunteers in the reference and test groups have similar characteristics.

Figure 2 indicates the relative changes in the parameters that showed changes during the intervention period in one or both groups. Changes were generally more pronounced for individuals consuming the n-3 PUFA-enriched tomato juice, which suggested possible synergistic action between n-3 PUFAS and natural tomato bioactive compounds. This effect was particularly evident in the case of the FRAP, homocysteine, and ICAM-1 levels, whose variations were around two-fold higher than those observed in the reference juice group. The FRAP values showed an 18% increase and a 23% increase for the reference juice and the n-3 PUFA-enriched juice groups, respectively. A 21% decrease was observed in the serum homocysteine concentrations following consumption of the enriched juice, whereas these levels remained essentially unchanged in the reference juice group. Similarly, ICAM-1 levels were weakly affected by reference tomato juice intake but showed a 24% decrease upon consumption of the enriched juice. Reference and n-3 PUFA-enriched juice intake decreased VCAM-1 levels by 42 and 47%, respectively.

Discussion

In the present study, we addressed the question of whether short-term consumption of tomato juice enriched with PUFAs could have a positive effect on serum lipids, levels of biomarkers of antioxidant status, and CVD risk. Serum lipid measurements indicated that intervention with the n-3 PUFA-enriched juice for 2 weeks failed to ameliorate the lipid profile of the volunteers. The likely reason for this lack of effect is the short duration of the study, as positive effects on blood lipids have generally been reported after longer intervention periods using comparable amounts of n-3 PUFAs. Several studies with n-3 PUFA supplemented milk showed significant changes in the plasmatic lipid profile, but these studies were carried out for periods ranging from 4 weeks to 1 year [36–38]. Despite the fact

that our intervention period could be considered too short, in light of these previous studies, a positive result on serum lipids was reasonably expected for several reasons: Firstly, Raghu and Venkatesan documented an improvement in the serum lipid profile after the intake of 660 mg EPA+DHA for 2 weeks [39]. They observed a significant increase in HDL and significant decreases in TGA, VLDL, LDL, and total cholesterol. Secondly, Jacob et al. [28] reported a significant decrease in total cholesterol after a 2-week intervention with plain tomato juice that provided 20 mg of lycopene per day. Thirdly, since both lycopene and n-3 PUFAs are able to inhibit the enzyme 3-hydroxy-3-methyl glutaryl coenzyme A reductase, the rate-limiting enzyme in cholesterol synthesis [1, 40], a decrease in cholesterol levels might have resulted from their combined action.

Nevertheless, data regarding the effect of n-3 PUFAs on blood lipids are sometimes contradictory. For example, Murphy et al. [15] reported that a daily intake of 1 g EPA+DHA for 6 months had no effect on serum lipids, whereas Schmidt et al.[41] reported that a supplementation with 4 g n-3 PUFA per day during 9 months could reduce TGA but did not change total or LDL cholesterol. Likewise, in the present study, supplementation with the n-3 PUFA-enriched juice did not elicit any change in CRP, a marker of inflammation that has been shown in multiple prospective epidemiological studies to predict incidence of myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death [42]. These results are in agreement with those reported in other studies [15, 38], despite inverse relationships between n-3 PUFAs and CRP levels previously described [43]. Longer supplementation periods (up to 1 year) were needed to significantly lower CRP levels [37].

Antioxidant status also showed improvement after the intervention period due to consumption of tomato juices. The amelioration of human antioxidant status after tomato consumption has been reported by others [28, 44], but the impact of n-3 PUFAs on the antioxidant status is often regarded as controversial. One generally expressed concern is that the increased intake of PUFAs may enhance lipid peroxidation, and that these oxidized products could in turn be harmful to tissues. However, increased intake of EPA/DHA has been reported to reduce in vivo lipid peroxidation and oxidative stress in humans [1, 36]. A positive association between the n-3 PUFA content of red blood cells, which reflects the consumption of n-3 PUFAs, and plasma antioxidant capacity, has been also described [45]. In the present study, the consumption of n-3 PUFA-enriched juice did not produce any increase in lipid peroxidation, since MDA levels remained unchanged during the intervention period. Only a weak variation in serum MDA was observed, which was more marked in the n-3 PUFA group and suggested that the combination of tomato antioxidants with n-3 PUFAs could have an enhancement effect. A similar behavior was observed for FRAP values, which showed a significant increase in the n-3 PUFA group. The enhancement of antioxidant defences has been reported in rats supplemented with fish oil rich in n-3 PUFAs. The improvement was revealed by the reduction of plasma peroxidation and the increase in the activity of antioxidant enzymes such as superoxide dismutase, glutathione peroxidases, and xanthine oxidase, as well as by a rise in plasma nitric oxide levels [46]. In conjunction, these data suggest that n-3 PUFAs may contribute to enhance the organism's antioxidant defences and that they seem to act synergistically with tomato antioxidants.

We provided a better characterization of the physiologic effects of n-3 PUFA-enriched juice consumption by evaluating parameters related to CVD risk. Among these, we focused on homocysteine, because epidemiological studies have shown that high blood concentrations of homocysteine (>15 μM) appear to be associated with higher risk of coronary, cerebral, and peripheral vascular disease and are inversely related to blood levels of folates and of vitamins B_{12} and B_6 [27, 47]. Even moderate hyperhomocysteinemia (>9 μM) is prospectively associated with an increased risk of mortality in CVD patients [48], and a 5 μM increase in plasma homocysteine concentration has been estimated to raise the risk of CVD by about 20% [49, 50]. In the present study, serum folates did not change as a result of the intervention in either juice group; however, the significant decrease observed in serum homocysteine would appear to be related to n-3 PUFA intake, since serum homocysteine only changed significantly in the n-3 PUFA group. This group showed a moderate hyperhomocysteinemia at time 0, and a 21% decrease was observed in homocysteinemia at the end of the intervention. In contrast, the intake of plain tomato juice did not change the initial values of this parameter in the reference group. The capability of n-3 PUFAs to lower circulating homocysteine has been observed in both animal [51] and human studies [52, 53], and the underlying mechanism for this effect seems to be the ability of n-3 PUFAs to regulate the activity of the enzymes involved in homocysteine metabolism [51].

Atherosclerosis and inflammation share similar mechanisms in their early phases, as each involves increased interactions between vascular endothelia and circulating monocytes. This process, so-called endothelial activation, involves the endothelial synthesis of VCAM-1 and ICAM-1 and is a key event in the onset of atherosclerosis [7, 52]. In this regard, several studies have suggested that plasma/serum levels of these adhesion molecules constitute good markers for long-term prediction of cardiovascular events [53]. The efficacy of n-3 PUFA supplementation in reducing the levels of vascular adhesion molecules has

been previously reported in human studies [36, 37, 54]. Consistent with this, we observed a significant reduction in VCAM-1 and ICAM-1 levels after short-term intervention with n-3 PUFA-enriched juice. Plain tomato juice consumption also had an effect on adhesion molecules, indicating that tomato antioxidants alone can also contribute to modulation of their circulating levels, particularly in the case of VCAM-1, where plain tomato juice consumption resulted in a 42% reduction. These changes in vascular adhesion molecules could indicate a synergistic effect between lycopene and n-3 PUFA in the reduction of the level of VCAM-1, whereas the level of ICAM-1 may only be modulated by the intake of n-3 PUFAs. The reduction in VCAM-1 associated with the presence of lycopene in tomato juice is in agreement with the data reported by Liu et al. [55] in hyperhomocysteinemic rats. In contrast, Blum et al. [56] showed no changes in plasma concentrations of ICAM-1 in healthy subjects supplemented with a tomato-rich diet, as occurred in the reference group in the present study.

Relationships between oxidative stress, homocysteine, and endothelial activation have also been proposed in a number of studies. Homocysteine has been demonstrated to promote reactive oxygen species production and LDL oxidation and to induce expression of adhesion molecules, thereby contributing to both oxidative damage and endothelial activation. Reactive oxygen species, free radicals, cytokines, and oxidized molecules (LDL, advanced glycation end products, etc.) also stimulate expression of adhesion molecules [7, 57–59].

In summary, short-term intake of n-3 PUFA-enriched tomato juice does not modify the serum lipid profile of healthy female individuals, but positive effects are observed for serum total antioxidant capacity, the serum levels of homocysteine and the levels of the adhesion molecules ICAM-1 and VCAM-1. In addition, synergistic effects are suggested to occur between n-3 PUFAs and natural tomato bioactive compounds. This possibility of in vivo synergistic action should be further investigated to ascertain the underlying molecular mechanisms.

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