A Mediterranean-style breakfast increases postprandial serum α-tocopherol levels in lean and obese individuals

S. García-Rodríguez[a], L. Sinausia[a], C. Barragán[a], E. Montero[b] and J.S. Perona[a,✉]

[a]Food and Health, Instituto de la Grasa-CSIC, Seville, Spain.
[b]Emergency unit, HHUU Virgen del Rocío, Seville, Spain.

✉Corresponding author: perona@ig.csic.es

SUMMARY: The aim of this study was to compare the variations in the concentrations of tocopherols and retinol in obese adults in the postprandial state after the intake of a Mediterranean or Western-style breakfast. The study was designed as a randomized, controlled intervention trial in the postprandial state, for which 24 male adults (12 obese and 12 of normalweight) were recruited. After a fat challenge, blood samples were collected at different times postprandially and α-tocopherol, γ-tocopherol and retinol concentrations were determined in serum by HPLC. The Mediterranean-style meal produced a greater increase in serum α-tocopherol levels in both obese and normal-weight subjects, compared to the Western-style meal, indicating that the composition of the food affects the concentration of tocopherols in the postprandial state. However, the serum concentrations of γ-tocopherol and retinol remained unmodified. In conclusion, the presence of α-tocopherol in the meal could contribute to the protection of the Mediterranean-style meal against atherosclerosis in the postprandial state.

KEYWORDS: *α-tocopherol; Breakfast; Mediterranean-style meal; Obesity; Postprandial; Western-style meal*

RESUMEN: ***El desayuno de estilo mediterráneo aumenta los niveles de α-tocoferol sérico posprandial en individuos delgados y obesos.*** El objetivo de este estudio fue comparar las variaciones en la concentración de tocoferoles y retinol en adultos obesos en el estado postprandial tras la ingesta de un desayuno mediterráneo u occidental. Consiste en un ensayo de intervención aleatorio y controlado, en el que participaron 24 adultos varones (12 obesos y 12 normopeso). Tras la ingesta de dichas comidas, se determinaron las concentraciones de α-tocoferol, γ-tocoferol y retinol en el suero de las muestras sanguíneas mediante HPLC. La comida de estilo mediterráneo aumentó los niveles séricos de α-tocoferol en sujetos obesos y de peso normal, en comparación con una comida de estilo occidental, lo que indica que la composición del alimento afecta a su concentración sérica. Sin embargo, referente al γ-tocoferol y retinol, permanecieron sin modificaciones. En conclusión, la presencia de α-tocoferol en una comida de estilo mediterráneo podría contribuir a la protección contra la aterosclerosis en el estado postprandial.

PALABRAS CLAVE: *α-tocoferol; Comida de estilo Mediterráneo; Comida Occidental; Desayuno; Obesidad; Postprandial*

ORCID ID: García-Rodríguez S https://orcid.org/0000-0003-2096-9655, Sinausia L https://orcid.org/0000-0002-7605-5310, Barragan C https://orcid.org/0000-0003-4622-2824, Montero E https://orcid.org/0000-0002-1751-3545, Perona JS https://orcid.org/0000-0001-5919-993X

1. INTRODUCTION

Obesity is considered one of the main public health concerns in Western countries as it gives rise to other conditions, such as diabetes or cardiovascular disease. Obesity has also been associated with systemic inflammation and increased oxidative stress, which lead to decreased α-tocopherol bioavailability, increasing the requirements for this vitamin in obese individuals (Traber *et al.*, 2017). α-Tocopherol is capable of protecting cell membranes and low density lipoproteins (LDL) from lipid peroxidation (Trpkovic *et al.*, 2015), an alteration that is necessary to trigger atherogenesis (Cabello-Moruno *et al.*, 2007). LDL from obese subjects are more susceptible to oxidation and thus, may require more antioxidants for protection against oxidative stress (Maruyama *et al.*, 2013). The possible relevance of postprandial α-tocopherol depletion and its relation with lipid peroxidation in diabetic individuals has been suggested (Manuel-Y-Keenoy *et al.*, 2004). In addition, it has been observed that the intake of antioxidant supplements, such as α-tocopherol, has a greater effect on oxidative stress in subjects with postprandial anomalies such as hyperglycemia and hyperlipidemia, which are very common in obese individuals (Plotnick *et al.*, 1997).

There is also considerable evidence that vitamin A homeostasis is involved in regulating body fat and blood glucose levels. It appears that altered levels of circulating retinol have a relevant role in the modulation of endocrine hormones and gene transcription associated to lipid and glucose metabolism (Mody 2017). Subjects with a higher adherence to the Mediterranean diet have higher plasma concentrations of β-carotene and α-tocopherol compared to other dietary patterns, whereas retinol levels remain unchanged (Bach-Faig *et al.*, 2006). However, the effect of a Mediterranean-style breakfast on postprandial serum concentrations of tocopherols and retinol has not been addressed to date.

This study was designed to determine whether plasma concentrations of retinol and tocopherols (α-tocopherol and γ-tocopherol) are altered in the postprandial state in obese individuals and whether a Mediterranean-type breakfast can contribute to restoring such concentrations.

2. MATERIALS AND METHODS

2.1. Subjects

Twenty-four adult male subjects (22-56 years-old), without a history of digestive or metabolic disorders, were recruited for the study by placing announcements in online social networks and among acquaintances of the research team in the city of Seville (Spain). Sample size was calculated by power analysis, considering a type 1 error (α) = 0.05 and a power (1-β) = 0.9 and the resulting minimum number was 10 individuals per group. Subjects were classified as normal-weight or obese using BMI cut-off points according to age and sex. Twelve adults were obese (BMI > 30 Kg/m^2) and 12 normal-weight (BMI = 20-25 Kg/m^2). A written consent form, which was approved by the Institutional Committee on Human Research of HHUU Virgen del Rocío (Seville, Spain), was obtained from the participants. The consent form and the protocol were in accordance with the institutional and national ethical standards for human experimentation and the Helsinki declaration of 1975 (revised in 2000). The study was registered in clinicaltrials.gov (NCT01518803).

2.2. Study design

The study was designed as crossed-over to test the effect of two variables, obesity and meal composition, on α-tocopherol, γ-tocopherol and retinol serum postprandial concentrations. Participants were asked to have a low-fat dinner the evening prior to the postprandial assay and to abstain from alcohol drinking and smoking for 24 h. After an overnight fast (12 h), a cubital vein was catheterized and a baseline blood sample was taken immediately before consumption of the first test meal, which was consumed in less than 15 minutes. Blood samples were collected at baseline (0h) and at 2h and 4h after the intake of the meals. During the course of the experiment, the subjects were allowed to drink water and to undertake only light activities. The subjects consumed a Mediterranean breakfast consisting of 3 slices of brown bread (71 g), 200 mL of skimmed milk, 250 mL of orange juice and 20 g of mashed tomato and 57 g of olive oil. The composition of the meal was selected to reflect the habitual breakfast consumption in southern Spain. Olive oil was kindly supplied by Oleicola El Tejar, S.A. (El Tejar, Cordoba, Spain). The Western breakfast consisted of 55 g of butter, three slices of brown bread and a glass of skimmmed milk (200 ml) with cocoa powder (20 g). The main composition of the meals is indicated in Table 1. The composition of α-tocopherol and retinol in olive oil and butter was determined as described below for serum and is depicted in Table 2. The olive oil was directly dissolved in hexane but the butter was first heated at 37 ºC to break the emulsion. The tocopherol and retinol contents of the other components of the meals were recorded from the Spanish database of food composition (Spanish Agency for Consumer Affairs, Food Safety and Nutrition).

2.3. Baseline biochemical analyses

Serum glucose, cholesterol and triglycerides were determined by enzymatic colorimetric methods on a Roche/Hitachi System analyzer (Roche Diagnostic, Mannheim, Germany). Serum HDL-cholesterol was measured by a direct enzymatic method (HDL-C-plus 2nd generation, Roche Diagnostics, Mannheim, Germany) on a Roche/Hitachi System analyzer (Roche Diagnostic) and LDL cholesterol was estimated by the Friedewald equation (Friedewald *et al.*, 1972). Insulin was measured by immunoassay (Abbott Laboratories, Maidenhead, UK) and the Homeostasis Model Assessment of the insulin resistance (HOMA-IR) score was calculated using the HOMA Calculator software (Diabetes Trial Unit, Churchill Hospital, Oxford, UK).

TABLE 1. Nutrient compositions of the experimental meals.

	Western breakfast	Mediterranean breakfast
Energy (kJ)	1963	2022
Protein (g)	14	15
Carbohydrates (g)	53	62
Sucrose (g)	26	26
Fats (g)	53	57
SFA (g)	32	8
MUFA (g)	15	44
PUFA (g)	2	5
Fiber (g)	6.4	7.0
Cholesterol (mg)	104	0
Tocopherols (mg)	1.70	45.85

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

2.4. Determination of tocopherols and retinol

Liposoluble vitamins were extracted from 500 µL of serum as follows: 500 µL of ethanol and 1mL of hexane were added sequentially. The mixture was centrifuged (3500 rpm, 15 ℃ for 10 min) and the supernatant was collected. Hexane was evaporated under a stream of nitrogen and the vitamins were re-dissolved in choloroform/methanol (2:1, v/v) for injection into the the HPLC system. This system consisted of a reversed-phase column (Novapack) and an elution system composed of methanol/acetonitrile (chloroform (9:78:13, v/v/v) which run isocratically. A photodiode array detector (PAD 996, Waters) was used for α-tocopherol, γ-tocopherol and retinol detection at 290 nm (α-tocopherol and γ-tocopherol) and 325 nm (retinol), respectively. These compounds were identified using commercial standards and quantified by means of external standard.

2.5. Statistical analysis

The results were expressed as mean ± SEM. Data analyses were performed with the GraphPad Prism[®] 5 statistical package (GraphPad Software Inc., San Diego, CA). The baseline statistical significance of anthropometric measurements, serum biochemical determinations and serum α-tocopherol, γ-tocopherol and retinol concentrations between obese subjects and their normalweight counterparts were assessed with the Student's t test. The same comparisons were made for the incremental AUC (iAUC) values which were calculated from the postprandial curves. A 2x2 ANCOVA test was applied on iAUC values using the meals (Mediterranean and Western) and body size as the independent variables. For those comparisons which resulted in significantly different, a Student's t test was used to compare the iAUC values. Differences were considered statistically significant at $p < 0.05$.

TABLE 2. α-Tocopherol and retinol equivalent compositions of the experimental meals.

	Western breakfast	Mediterranean breakfast	Western breakfast	Mediterranean breakfast
	α-Tocopherol (mg)		Retinol (µg)	
Brown bread	0.47	0.47	ND	ND
Skimmed milk	0.02	0.02	ND	ND
Orange juice	-	0.43	-	182.5
Tomato	-	0.18	-	16.4
Olive oil	-	44.75	-	19.4
Cocoa powder	0.11	-	0.2	-
Butter	1.10	-	430.7	-
Total	1.70	45.85	430.9	218.3

ND: not detected. - : ingredient not present in the meal.

3. RESULTS

3.1. Anthropometric characteristics and serum lipid, glucose, insulin and adipokine concentrations

Table 3 shows the anthropometric characteristics and fasting serum concentrations of lipids, glucose, insulin and liposoluble vitamins. Compared to their normal-weight subjects, obese individuals presented higher values of BMI, serum triglycerides, total and LDL-cholesterol, glucose, and systolic pressure values, but not diastolic blood pressure. In addition, this group presented lower HDL-cholesterol concentrations. No significant difference was observed for baseline serum concentrations of α-tocopherol and γ-tocopherol but the retinol content was higher in the obese group.

3.2. Postprandial concentrations of α-tocopherol and γ-tocopherol and retinol

The postprandial concentrations of the liposoluble vitamins analyzed are depicted in Figure 1. The values were normalized with the concentrations at 0h. For all compounds, except for γ-tocopherol in the obese group, significant differences were observed 4h after the intake of the meals but not at 2h. The Mediterranean-style breakfast increased α-tocopherol concentrations at 4h postprandially in both groups (Figures 1A and 1B). In contrast, this breakfast reduced the content of γ-tocopherol (Figure 1C) and retinol (Figure 1E) at that time

point compared with the Western-style meal but only in the normalweight group. In obese subjects, no differences were found for γ-tocopherol between meals (Figure 1D) and the Mediterranean meal increased the content of retinol (Figure 1F).

As shown in Figure 2, α-tocopherol postprandial iAUC values were significantly higher in both groups after the intake of the Mediterranean meal (Figure 2A and 2B). In fact, α-tocopherol values were affected by the meal only, and not by body size (Table 4). In contrast, no significant differences were observed in iAUC values for γ-tocopherol and retinol after the intake of the experimental meals (Figures 2C, 2D, 2E and 2F). These values were not affected by any of the two variables studied (meal and body size) according to 2X2 ANCOVA (Table 4).

4. DISCUSSION

We report here that a Mediterranean-style meal can increase postprandial serum α-tocopherol levels in both obese and normal weight subjects compared to a Western-style meal. The content of α-tocopherol was higher in the Mediterranean breakfast, mainly due to its content in olive oil (Table 2), but also to orange juice and tomato. Ascorbic acid is also present in orange juice and tomato but its role in protecting α-tocopherol from oxidation is still controversial. Porkkala-Sarataho et al., (1996) reported that in contrast to α-tocopherol, supplementation with ascorbic acid did not have any significant

TABLE 3.   Anthropometric characteristics and baseline serum concentrations of lipids, glucose, insulin and liposoluble vitamins.

	Normal-weight	Obese	p
Age (y)	27.3 ± 2.9	35.8 ± 3.5	0.075
Weight (kg)	74.9 ± 2.3	112.9 ± 3.9	<0.001
Height (cm)	179.6 ± 2.0	180.8 ± 1.5	0.778
BMI (kg/cm^2)	23.2 ± 0.3	34.7 ± 1.4	<0.001
Systolic Pressure (mmHg)	110.9 ± 3.7	130.8 ± 2.2	<0.001
Diastolic Pressure (mmHg)	68.9 ± 2.1	74.5 ± 2.9	0.120
Glucose (mg/dL)	73.7 ± 1.4	83.6 ± 2.4	0.002
Insulin (mU/ml)	4.9 ± 0.8	12.6 ± 1.2	<0.001
Triglycerides (mg/dl)	78.7 ± 7.1	129.7 ± 17.4	0.013
Cholesterol (mg/dl)	172.5 ± 9.4	202.9 ± 10.0	0.030
LDL-c (mg/dl)	94.7 ± 5.6	158.7 ± 4.8	<0.001
HDL-c (mg/dl)	57.5 ± 2.8	45.9 ± 2.5	0.005
α-Tocopherol (µg/ml)	9.87 ± 0.73	10.48 ±1.69	0.703
γ-tocopherol (µg/ml)	0.17 ± 0.03	0.14 ± 0.02	0.414
Retinol (µg/ml)	0.45 ± 0.04	0.73 ± 0.10	0.016

Data are expressed as mean ± SEM; n = 12. BMI: body mass index. LDL-c, low-density-lipoprotein cholesterol; HDL-c, high-density-lipoprotein cholesterol. Statistical differences were assessed by the Student's t test.
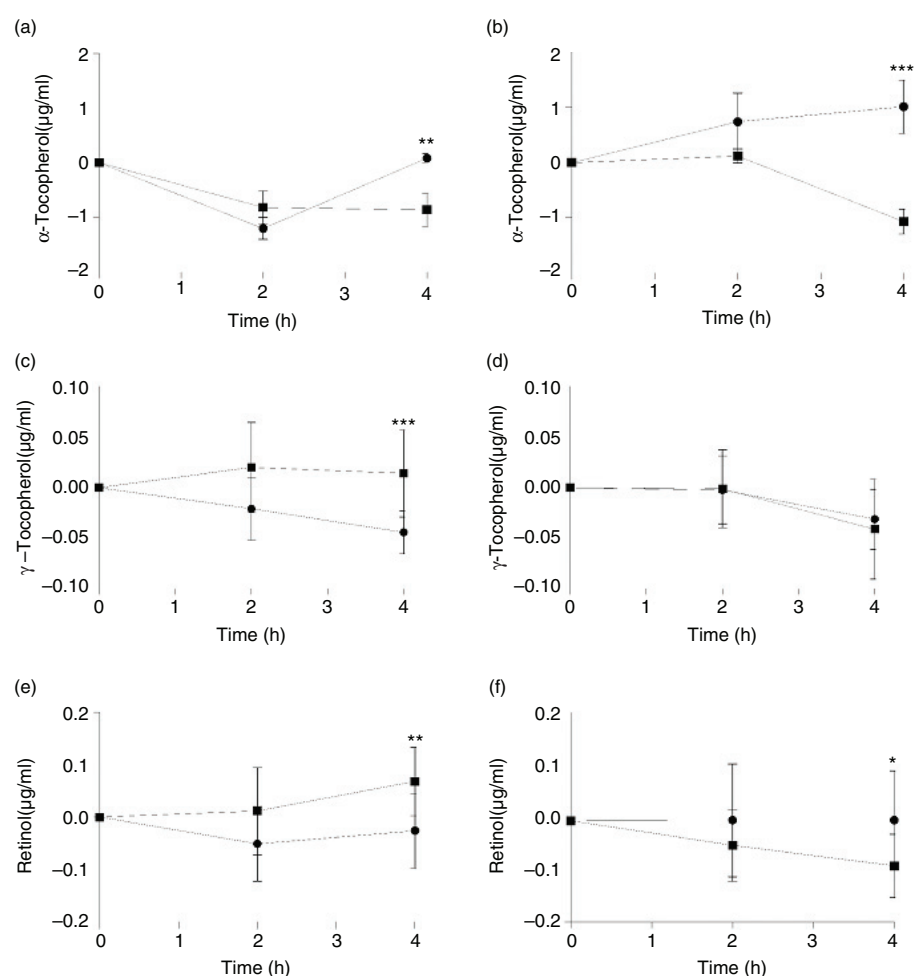
FIGURE 1. Serum postprandial α-tocopherol, γ-tocopherol and retinol concentrations in the normal weight (1a, 1c and 1e) and obese (1b, 1d and 1f) groups in the postprandial state. Values corresponding to the Mediterranean meal are represented by circle dots, whereas values corresponding to the Western meal are represented by square dots. Data are shown as mean ± SEM. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

effect on VLDL and LDL oxidation, which was confirmed more recently by Van Hoydonck et al., (2004). However, Uzun et al., (2013) demonstrated that vitamin C or its combination with vitamin E significantly increased endothelial vasodilation in patients with coronary artery disease. Therefore, dietary supplementation with ascorbic acid might not have an impact on α-tocopherol plasma levels, but they might act synergistically. This can also be the case of lycopene, which is found in relevant concentrations in tomato. Furhman et al., (2000) found that a combination of lycopene and α-tocopherol showed a greater inhibition of LDL oxidation, compared to the compounds tested alone, confirming the synergistic effect.

Traber et al., (2015) reported that blood concentrations of α-tocopherol are associated with the mechanisms which control lipid concentrations. These authors stated that postprandial α-tocopherol remains in circulation for a longer time as the concentration of serum lipids increases, and that lipoprotein clearance is retarded in obese individuals. Very recently, these authors showed that subjects with metabolic syndrome displayed higher levels of biomarkers of oxidative stress and inflammation in plasma, suggesting that α-tocopherol requirements had increased (Traber et al., 2017).

In our study, significant differences in plasma α-tocopherol concentrations between meals were found only at 4 hours postprandially. At this time point, the size of triglyceride-rich lipoproteins (TRL), which are in fact the transporters of tocopherols and retinol, is key in atherogenesis (Amigo-Benavent et al., 2016). Cabello-Moruno et al., (2014)
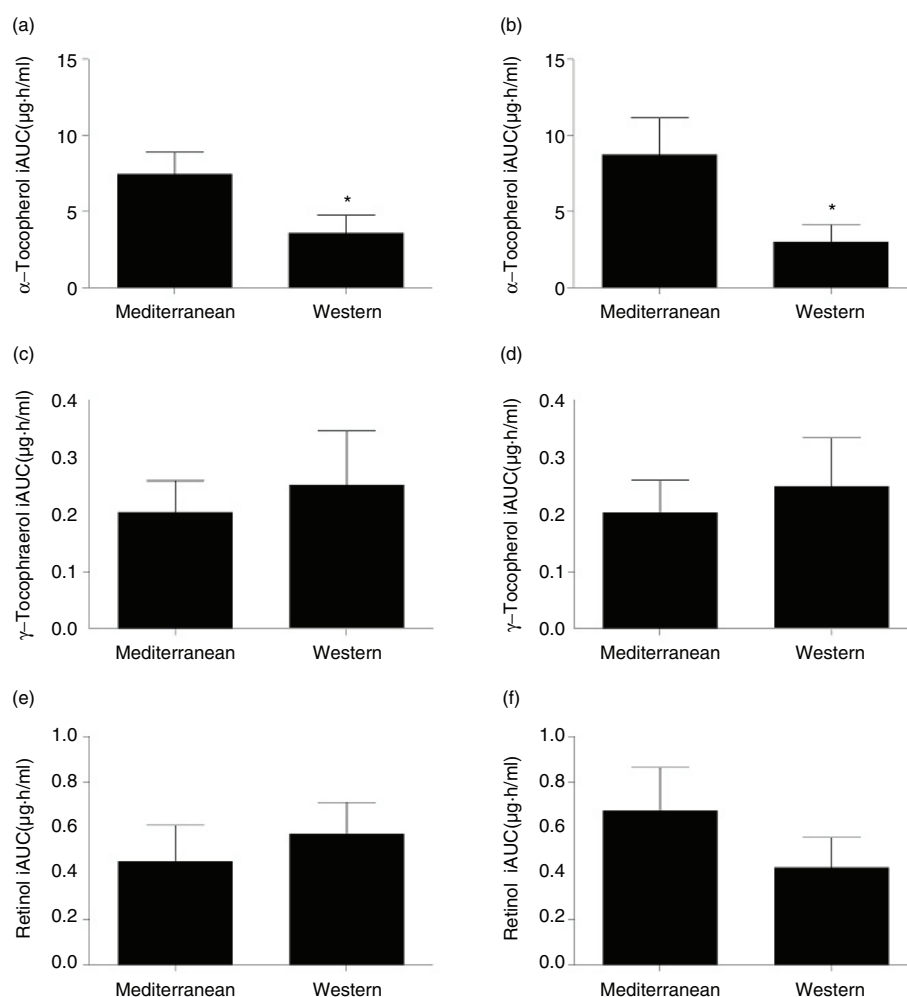
FIGURE 2. Incremental area under the curve (iAUC) values for serum postprandial α-tocopherol, γ-tocopherol and retinol concentrations in the normal weight (2a, 2c and 2e) and obese (2b, 2d and 2f) groups. Data are shown as mean ± SEM.
*: p < 0.05.

TABLE 4. Impact of body size, type of breakfast and their interaction on the postprandial incremental area under the curve (iAUC) values of the study endpoints.

	Body size			Breakfast			Body size x breakfast		
	F	p	Partial ETA square	F	p	Partial ETA square	F	p	Partial ETA square
α-Tocopherol	0.105	0.747	0.003	7.252	0.011	0.164	0.429	0.517	0.011
γ-Tocopherol	0.001	0.980	0.000	0.401	0.531	0.011	0.000	0.988	0.000
Retinol	0.059	0.809	0.001	0.173	0.680	0.004	1.396	0.244	0.034

2x2 ANCOVA was applied on iAUC calculated from the postprandial profiles for each dependent variable, using body size (normal weight or obese) and breakfast (Mediterranean or Western) as independent variables. Partial ETA square was used to measure the effect size of the variable.

observed that triglyceride accumulation was greater when the cells were incubated with the postprandial TRL of intermediate size which were isolated at 4 h after a lipid load. In this regard, Armengol-Lopez et al., (2012) reported that chylomicron remnants (CMR), the most abundant TRL present in plasma at 4 h postprandially, are pro-inflammatory and are linked to atherogenic signalling in primary human monocytes. This implies an increased secretion of chemokines and cytokines, together with the production of the reactive oxygen species (ROS), which can be mitigated by α-tocopherol (Godbout et al., 2004).

In addition, α-tocopherol has been shown to reduce the expression of scavenger receptors in macrophages (Teupser *et al.*, 1999), which are essential for CMR uptake (Cabello-Moruno *et al.*, 2014).

Therefore, we believe that postprandial α-tocopherol may play a protective role in serum against atherosclerosis and that the Mediterranean breakfast favors it positively. In this regard, Carnevale *et al.*, (2014) showed that when extra virgin olive oil was included in a Mediterranean diet, it significantly reduced postprandial oxidative stress and blocked the decrease in serum levels of vitamin E, which is in agreement with our findings.

Nielsen *et al.*, (2000), studied the effects in the postprandial state of two experimental meals containing different sources of dietary fats (sunflower oil, rapeseed oil, olive oil, palm oil or butter). They found that the postprandial plasma content of α-tocopherol was significantly higher after the meal rich in olive and sunflower oils compared to the meal rich in palm oil but the γ-tocopherol content in VLDL was higher after the rapeseed oil meal. We did not find significant differences in serum γ-tocopherol concentrations after the consumption of the Mediterranean or Western breakfasts. Bates *et al.*, (2004) reported strong correlations between higher plasma γ-tocopherol levels and obesity indexes, such as body weight, BMI and waisthip ratio. In addition, Hak *et al.*, (2003) showed that men with high plasma γ-tocopherol levels were more likely to have an increased risk of myocardial infarction. Therefore, it seems that, in contrast to α-tocopherol, higher plasma γ-tocopherol levels do not exert a protective or favorable effect against atherosclerosis. However, others have suggested that in healthy individuals, γ-tocopherol supplementation maintains vascular endothelial function during postprandial hyperglycemia, possibly attenuating lipid peroxidation (Mah *et al.*, 2013).

We also were unable to find differences in serum postprandial retinol concentrations between the groups when the results were expressed as iAUC. Guerci *et al.*, (2000), found reduced plasma retinyl palmitate at 4 h postprandially in obese individuals after an oral lipid challenge. They argued that since retinyl palmitate is considered a marker for intestinal TG-rich lipoproteins, the lower concentrations observed were predominantly due to CMR. Nevertheless, the lack of significant differences found in the present study for retinol is not surprising because the levels of this biomolecule are highly regulated and vary little with food intake, except in cases of supplementation or certain pathologies (Ascherio *et al.*, 1992; Hak *et al.*, 2003).

The 2x2 ANCOVA test employed in the present study showed that the type of breakfast affected the levels of α-tocopherol in plasma but no influence of body size on the postprandial levels of tocopherols

and retinol. Although, as stated above, Bates *et al.*, (2004) reported that γ-tocopherol levels were related to obesity, correlations were weak in young individuals like the ones enrolled in our study. More importantly, they did not have postprandial data. In fact, to our knowledge, our study is the first one to report postprandial γ-tocopherol levels in obese individuals.

5. CONCLUSIONS

In conclusion, a Mediterranean-style breakfast increases serum α-tocopherol, but not γ-tocopherol or retinol content in both lean and obese individuals in the postprandial state compared to a Western-style meal. Interestingly, the highest α-tocopherol concentration was found 4h after the meal intake, a time point at which CMR are most atherogenic. The well-known antioxidant properties of α-tocopherol together with its reported abilities to down-regulate scavenger receptors in macrophages, could contribute to the protection of the Mediterranean-style meal against atherosclerosis in the postprandial state.

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