

α -Tocopherol Stereoisomers in Human Plasma Are Affected by the Level and Form of the Vitamin E Supplement Used^{1–3}

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Abstract

Background: Studies examining vitamin E intake and the percentage of the population meeting dietary guidelines do not distinguish between natural (*RRR*- α -tocopherol) and synthetic (*all-rac*- α -tocopherol) intake, even though these different isomeric forms differ in bioactivity.

Objective: This study aimed to determine the effect of *RRR*- α -tocopherol vs. *all-rac*- α -tocopherol intake on the percentage of the population meeting the vitamin E recommendation and on plasma α -tocopherol stereoisomer distribution.

Methods: With the use of data from the Irish National Adult Nutrition Survey (NANS), this study examined the percentage of the Irish population meeting the European Union (EU) RDA for vitamin E of 12 mg/d, correcting for a bioactivity difference in *all-rac*- vs. *RRR*- α -tocopherol, where 1 mg of *all-rac*- α -tocopherol is considered to be equivalent to 1:1.36 (0.74) mg in the EU RDA. In a subcohort of supplement users and nonusers, plasma α - and γ -tocopherol concentrations and α -tocopherol stereoisomer distribution were measured. Receiver operating characteristic (ROC) curve analysis was conducted to determine ability to discriminate supplement user types.

Results: Analysis of the NANS showed that 100% of participants still met the recommended intake of 12 mg/d, after *all-rac*- α -tocopherol intake was corrected for α -tocopherol equivalent bioactivity. In the subcohort analysis, the percentage of plasma *RRR*- α -tocopherol was significantly lower in high *all-rac*- α -tocopherol supplement (>11 mg/d) users (82%) compared with nonusers and with high *RRR*- α -tocopherol supplement (>35 mg/d) users (91% and 93% respectively, $P < 0.01$). High *RRR*- α -tocopherol supplement users had a significantly higher plasma α -tocopherol than low *all-rac*- α -tocopherol supplement (<2.5 mg/d) users (34 vs. 25 μ mol/L, $P = 0.01$). ROC analysis demonstrated an ability to distinguish between *RRR*- and *all-rac*- α -tocopherol consumers, which may be useful in investigating the potential effect of *RRR*- and *all-rac*- α -tocopherol intake on health.

Conclusions: This study demonstrated that the percentage of the population meeting the vitamin E recommendation was unaffected when *all-rac*- α -tocopherol intake was corrected for α -tocopherol equivalent bioactivity. *all-rac*- α -Tocopherol intake led to a decrease in the percentage of plasma *RRR*- α -tocopherol relative to *RRR*- α -tocopherol intake. *J Nutr* 2015;145:2347–54.

Keywords: vitamin E supplement intake, RDA, *RRR*- α -tocopherol, *all-rac*- α -tocopherol, bioactivity, plasma α -tocopherol stereoisomers, ROC analysis

Introduction

Vitamin E comprises α -, β -, γ -, and δ -tocopherol and 4 corresponding unsaturated analogs (α -, β -, γ -, and δ -tocotrienol)

(1). α - and γ -Tocopherol are the predominant forms of vitamin E consumed in the diet, of which α -tocopherol has greater antioxidant activity and is the main form circulating in the blood (2). *RRR*- α -tocopherol exists in natural food sources such as vegetable oils, seeds, and nuts (3), whereas synthetic α -tocopherol (*all-rac*- α -tocopherol) is composed of equal proportions of all 8 stereoisomers (*RRR*-, *RRS*-, *RSR*-, *RSS*-, *SRR*-, *SRS*-, *SSR*-, and *SSS*-) and is generally consumed as a dietary supplement (4).

Although all forms of vitamin E are absorbed without preference in the intestine and secreted into chylomicrons in proportions similar to those naturally occurring in the diet (5, 6),

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³ Supplemental Tables 1–3 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

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the incorporation of vitamin E into VLDL in the liver determines plasma vitamin E concentrations (7). Vitamin E is transported from the liver to various tissues via the hepatic α -tocopherol transfer protein, which preferentially incorporates RRR- α -tocopherol compared with other stereoisomers into VLDL for circulation (8). There are also known variations in retention and excretion, with Kiyose et al. (9) demonstrating a preferential retention of 2R-stereoisomers (RRR-, RRS-, RSR-, and RSS-) and elimination of 2S-stereoisomers (SRR-, SRS-, SSR-, and SSS-) in humans. Additionally, there is evidence to suggest variation among the stereoisomers with respect to bioavailability and bioactivity (10). A previous study found that the bioavailability ratio of *all-rac*- to RRR- α -tocopherol in human plasma was close to 1:2 (11). With the use of the rat fetal resorption assay, the bioactivity ratio of *all-rac*- to RRR- α -tocopherol was determined to be 1:1.36 (12).

It has been established that only α -tocopherol prevents the vitamin E deficiency disorder ataxia (13); therefore, the US Institute of Medicine Estimated Average Requirement (IOM-EAR)⁴ considers only α -tocopherol, which includes the naturally occurring form (RRR- α -tocopherol) and the other 3 synthetic 2R-stereoisomeric forms of *all-rac*- α -tocopherol when estimating vitamin E intake (13). This estimation is aligned to an equivalent bioavailability dose ratio of 1:2 (*all-rac*- to RRR- α -tocopherol). However, in contrast to the IOM-EAR, the European Union (EU) RDA takes total vitamin E intake into account and expresses vitamin E intake in α -tocopherol equivalents, where, for conversion, 1 mg of *all-rac*- α -tocopherol should be multiplied by 0.74 (corresponding to the previously mentioned bioactivity ratio of 1:1.36), β -tocopherol should be multiplied by 0.5, γ -tocopherol by 0.1, and α -tocotrienol by 0.3 (14). Both the EU RDA (14) and IOM-EAR are set at 12 mg/d (15); however, with the differences in definitions and conversion indexes used in assessing intake of vitamin E between the European Union (14) and the United States (13), comparisons of intake and estimations of populations meeting recommended intake across the 2 jurisdictions can lead to confusion.

To date, the impact of *all-rac*- α -tocopherol consumption on the percentage of the population meeting the recommended vitamin E intake has not been discussed. In addition, the influence of RRR- and *all-rac*- α -tocopherol in the diet on plasma α - and γ -tocopherol concentrations and plasma α -tocopherol stereoisomers remains unknown. Furthermore, there may be potential for plasma α - and γ -tocopherol concentrations and distribution of α -tocopherol stereoisomers to be useful predictors in identifying RRR- vs. *all-rac*- α -tocopherol consumers.

The aim of the study was to quantify total vitamin E intake as α -tocopherol equivalents in Irish adults and to estimate the percentage of the population meeting the EU RDA, after correction for *all-rac*- α -tocopherol intake. The study also evaluated the effect of RRR- and *all-rac*- α -tocopherol intake on plasma α - and γ -tocopherol concentrations and α -tocopherol stereoisomer distribution in the subcohort of participants and attempted to assign the form of α -tocopherol (RRR- or *all-rac*- α -tocopherol) consumption with the use of plasma measurements as predictors.

Methods

Study population. The Irish National Adult Nutrition Survey (NANS) 2008–2010 was a cross-sectional dietary survey carried out in 1500

healthy adults (740 men and 760 women) aged 18 y or older from the Republic of Ireland during the years 2008–2010; 1129 participants provided blood samples (16). The fieldwork phase of the NANS was carried out between October 2008 and April 2010, providing a seasonal balance to the data. Ethical approval for the survey was obtained from the Human Ethics Research Committee of University College Dublin and the University College Cork Clinical Research Ethics Committee of the Cork Teaching Hospitals. Participants who agreed to take part signed a written consent form in accordance with the Declaration of Helsinki. The participants were representative of Irish adults with respect to age, gender, and social class distribution (Supplemental Table 1). A more detailed description of the sampling procedures and the survey methodology has been published elsewhere (16, 17). Among the 1129 participants who donated a blood sample, 420 subjects who were considered to have under-reported energy intake with the Goldberg method (18) were excluded, leaving a sample of $n = 709$ for estimating the percentage of the population meeting the recommended intake. In addition, for the purpose of this paper, 70 age- and gender-matched participants (20 nonusers of supplements and 50 supplement users) based on their vitamin E supplement intake level and form were selected from the NANS for analysis of vitamin E intake, plasma α - and γ -tocopherol concentrations, and α -tocopherol stereoisomer distribution.

Food composition data. Participants were asked to record detailed information regarding the amount and types of food and beverages and nutritional supplements consumed over 4 consecutive survey days. Participants were encouraged to weigh as many foods as possible and to ensure accuracy of recording, and a researcher visited participants in their home or workplace to demonstrate and check completion of the food diary during the recording period. The Irish National Food Ingredient Database version 3.0 was used to incorporate details of branded food, beverages, and nutritional supplements consumed during the NANS (19). Participants recorded brands and recipes, with details on cooking methods (including oils used). Food and nutritional supplement intake was analyzed with the use of WISP V3.0 (Tinuviel Software), which used data from McCance and Widdowson's "The Composition of Food," fifth and sixth editions, plus all 9 supplemental volumes (20–31). Modifications of the food composition database were made to include recipes of composite dishes, nutritional supplements, generic Irish food that was commonly consumed, and new food on the market.

Estimation of vitamin E intake and form from food and supplement sources. To estimate the vitamin E intake of Irish adults, all forms of vitamin E intake were taken into account based on EU guidelines (14), hereafter referred to as α -tocopherol equivalents throughout the paper. Three nutrient descriptors were used and will be referred to throughout the paper in the following manner: 1) all sources—vitamin E from foods and vitamin E from supplements; 2) food sources—vitamin E from foods only; and 3) supplements—vitamin E from supplements only. Information on α -tocopherol intake level and form from supplements was checked directly on the packaging and the manufacturers' websites. Within the NANS survey, only 0.7% of foods (18 from a total of 2552 foods) were found to be fortified with vitamin E. Therefore, foods fortified with vitamin E were not considered separately in the present study.

To evaluate the varied impact of RRR- and *all-rac*- α -tocopherol intake, α -tocopherol (RRR- and *all-rac*- α -tocopherol) supplement users and nonusers were selected. Within the subcohort, only supplements that clearly stated that α -tocopherol was one of active ingredients were included. Participants consuming supplements indicating "vitamin E" or "tocopherol" as one of ingredients, and not specifying the isomeric forms, were not selected. Four categories of supplement intake level and form were used to characterize intake of α -tocopherol stereoisomers in the subcohort, and these are referred to throughout the paper in the following manner: 1) nonusers ($n = 20$)— α -tocopherol intake only from foods; 2) low *all-rac*- α -tocopherol supplement users ($n = 20$)—supplements providing <2.5 mg/d *all-rac*- α -tocopherol; 3) high *all-rac*- α -tocopherol supplement users ($n = 20$)—supplements providing >11.0 mg/d *all-rac*- α -tocopherol; and 4) high RRR- α -tocopherol supplement users ($n = 10$)—supplements providing >35.0 mg/d RRR- α -tocopherol. A category of low RRR- α -tocopherol supplement users was not created, because intake in this category was found to be similar to that of nonusers.

⁴ Abbreviations used: EU, European Union; IOM-EAR, Institute of Medicine Estimated Average Requirement; NANS, Irish National Adult Nutrition Survey; ROC, receiver operating characteristic.

Blood sampling and biochemical analysis. The blood collection protocol used in the NANS has been described elsewhere (32). Blood processing and sample fractionations were performed at biological laboratories at University College Dublin and University College Cork and samples were stored at -80°C before analysis. All plasma samples were collected in tubes containing EDTA. Plasma α - and γ -tocopherol concentrations were determined by reversed-phase HPLC according to a modification of the method by Siluk et al. (33). A 20 μL aliquot of the methanol extract was analyzed on an Agilent 1200 series HPLC (Agilent Technologies). Separation was achieved on a Zorbax Eclipse XDB-C18 column (150 mm \times 4 mm i.d.; 5 μm particle size) with an RX-C8 guard column (12.5 mm \times 4.6 mm i.d.; 5 μm particle size). The column was attached to a 1200 series diode array detector (Agilent Technologies), and set at a wavelength of $\lambda = 295$ nm for α -tocopherol and γ -tocopherol and $\lambda = 298$ nm for α -tocopherol acetate (33). The mobile phase was 100% methanol at a flow rate of 1 mL/min.

The distribution of *RRR*-, *RRS*-, *RSR*-, *RSS*-, and 2*S*- α -tocopherol was determined as follows: α -tocopherol was extracted and separated as described above, collected with the use of an Agilent 1200 series fraction collector (Agilent Technologies), and derivatized following the method of Röhrle et al. (34). The separation of stereoisomers was achieved on a Diacel Chiralcel OD-H column (250 mm \times 4.6 mm i.d.; 5 μm particle size, absorbent cellulose derivatized with 3,5-dimethyl phenyl carbamate). The column was attached to an Agilent 1260 series fluorescence detector (Agilent Technologies) set at an excitation wavelength of 284 nm and emission of 326 nm for α -tocopherol stereoisomers. The mobile phase used was 100% hexane at a flow rate of 1 mL/min. Stereoisomer distribution was expressed as a percentage of total peak area for each stereoisomer and plasma concentration of *RRR*- α -tocopherol was derived from plasma α -tocopherol concentration and *RRR*- α -tocopherol distribution.

Statistical analyses. Statistical analyses were conducted with the use of SPSS version 20.0. The percentage of Irish adults meeting the existing EU RDA (14) was estimated by the method of Wearne and Day (35). Among the 70 selected participants, the plasma analyses of 3 participants (one nonuser, one low *all-rac*- α -tocopherol supplement user, and one high *all-rac*- α -tocopherol supplement user) were considered to be outliers, leaving 67 participants for all other analyses in the paper. One-factor ANOVA and chi-square test analyses were performed to compare the data from different categories of participants. Vitamin E intake from all sources, food sources alone, and supplement sources and plasma α - and γ -tocopherol concentrations and α -tocopherol stereoisomer distribution were log-transformed and compared across categories with the use of 1-factor ANOVA and Tukey's post hoc tests. Statistical significance was defined as $P < 0.05$.

Receiver operating characteristic (ROC) curve analysis was performed with the use of the ROCET program (36). In an attempt to distinguish α -tocopherol stereoisomers consumed or the extent of intake (high vs. low), the ability of plasma concentrations of α - and γ -tocopherol alone or in combination with the percentage of plasma stereoisomers was examined with the use of ROC analysis. First, we aimed to distinguish between supplement users, $n = 48$ (including 10 high *RRR*- α -tocopherol, 19 low, and 19 high *all-rac*- α -tocopherol supplement users) and nonusers, $n = 19$. Second, because we were interested in discriminating between

RRR- and *all-rac*- α -tocopherol consumption, the population was split into *RRR*- α -tocopherol consumers, $n = 29$ (which consisted of 10 high *RRR*- α -tocopherol supplement users and 19 nonusers), who only consumed *RRR*- α -tocopherol in their diet, and *all-rac*- α -tocopherol supplement users, $n = 38$ (19 low and 19 high *all-rac*- α -tocopherol supplement users), who consumed both *RRR*- and *all-rac*- α -tocopherol in the diet. Finally, we examined whether the predictors could distinguish between *RRR*- α -tocopherol supplement users (high *RRR*- α -tocopherol supplement users, $n = 10$) and *all-rac*- α -tocopherol supplement users ($n = 38$). When conducting ROC analysis with plasma concentrations of α - and γ -tocopherol in combination with the percentage of plasma stereoisomers, predictors were only included in this analysis if a significant difference was observed between categories with the use of 1-factor ANOVA (Supplemental Tables 2 and 3). The classification performance of the predictors was assessed by AUC.

Results

The percentage of NANS population meeting vitamin E EU RDA with *all-rac*- α -tocopherol correction. The proportion of Irish men and women meeting the EU RDA for vitamin E intake from food and all sources was considered (14) (Table 1). At the recommended intake of 12 mg/d, 100% of men and women achieved this intake from all sources, when *all-rac*- α -tocopherol intake was not corrected for a bioactivity ratio of 0.74. When *all-rac*- α -tocopherol intake from supplements was adjusted with the use of a *all-rac*- to *RRR*- α -tocopherol bioactivity ratio (0.74), the percentage of men and women meeting the vitamin E recommendation from all sources remained at 100%. When the intake from food sources was considered, only 68.4% of women met the population recommended intake, with a mean intake of 10.2 mg/d.

Characteristics and vitamin E intake of participants of different supplement intake categories. Vitamin E intake range (α -tocopherol equivalents) and demographic profiles of 67 adults from 4 different categories selected from the NANS subcohort are presented in Table 2. There were no significant differences in age, BMI, gender, or social class across the 4 supplement intake categories. All participants were nonsmokers, except for 3 (15.8%) in the low *all-rac*- α -tocopherol supplement users category. The results presented here were unaffected by the inclusion of smokers in this analysis.

Mean daily vitamin E intake (α -tocopherol equivalents) from all sources, food sources, and supplement sources of different supplement intake categories are presented in Table 3. Intake from all sources differed across categories ($P < 0.01$), with high *RRR*- α -tocopherol supplement users having a significantly higher intake at 126 ± 150 mg/d compared with the other 3 categories. High *RRR*- α -tocopherol supplement users had a significantly higher intake from supplements of 113 mg/d, whereas low and

TABLE 1 Daily vitamin E intakes of Irish adults and the percentage of the population meeting the recommended intake of 12 mg/d¹

	Vitamin E intake from food sources		Vitamin E intake from all sources ²			
	Natural sources		Natural sources plus synthetic sources		Natural sources plus synthetic sources \times 0.74 ³	
	Intake, mg α -tocopherol equivalents/d	% Population	Intake, mg α -tocopherol equivalents/d	% Population	Intake, mg α -tocopherol equivalents/d	% Population
Men	12.0 \pm 5.6	99.2	22.3 \pm 14.0	100	13.5 \pm 9.2	100
Women	10.2 \pm 4.2	68.4	15.8 \pm 39.3	100	15.5 \pm 39.2	100

¹ Values are means \pm SDs, $n = 361$ men and 348 women. European Union RDA of 12 mg/d vitamin E (14).

² Includes intake from both food and supplement sources.

³ Intake from supplements containing synthetic (*all-rac*- α -tocopherol) multiplied by 0.74.

TABLE 2 Demographics of Irish National Adult Nutrition Survey participants categorized on the basis of supplement intake level and form (synthetic or natural)¹

	Nonusers (n = 19)	Low <i>all-rac</i> - α -tocopherol supplement users (n = 19)	High <i>all-rac</i> - α -tocopherol supplement users (n = 19)	High <i>RRR</i> - α -tocopherol supplement users (n = 10)	P
Vitamin E, ² mg/dose		0.2–10	12.7–268	16–268	
Age, y	45.0 \pm 18.0	46.3 \pm 20.5	47.1 \pm 19.1	49.4 \pm 10.5	0.94
BMI, kg/m ²	27.3 \pm 5.3	25.1 \pm 4.5	26.6 \pm 3.8	29.4 \pm 7.3	0.20
Women	57.9	63.2	68.4	80.0	0.67
Smokers	0	15.8	0	0	0.05
Social class					0.65
Nonmanual skilled	11.1	5.3	26.3	33.3	
Semimanual skilled	22.2	26.3	21.0	11.1	
Manual skilled	16.7	15.8	10.5	0	
Professional	50.0	52.6	42.1	55.6	

¹ Values are means \pm SDs or percentages unless otherwise indicated. Synthetic supplements contain *all-rac*- α -tocopherol; natural supplements contain *RRR*- α -tocopherol.

² Range in supplement dose, as per manufacturer's report, consumed within each category.

high *all-rac*- α -tocopherol supplement users received significantly lower vitamin E from supplements (0.7 and 22.1 mg/d, respectively). There was no significant difference in intake from food sources observed across those categories ($P = 0.19$).

Plasma α -tocopherol stereoisomer distribution of participants of different supplement intake categories. α -Tocopherol stereoisomers were found to have different distributions in plasma across the 4 supplement intake categories (Table 4). The percentage of *RRR*- α -tocopherol in the plasma of high *all-rac*- α -tocopherol supplement users was 82.0%, significantly lower than nonusers and high *RRR*- α -tocopherol supplement users at 91.5% and 93.1%, respectively ($P < 0.01$). In the case of plasma percentages of *RRS*- and *RSS*- α -tocopherol, low and high *all-rac*- α -tocopherol supplement users had significantly higher percentages than those of high *RRR*- α -tocopherol supplement users ($P < 0.01$). In the case of *RSR*- α -tocopherol, high *all-rac*- α -tocopherol supplement users had a significantly higher percentage (5.9%) than all other supplement consumption categories ($P < 0.01$). Plasma percentage of the 2*S*-stereoisomers was not significantly different among categories ($P = 0.12$).

Plasma α -tocopherol concentrations of participants of different supplement intake categories. There were significant differences in plasma α -, *RRR*- α -, and γ -tocopherol concentrations across supplement intake categories, as shown in Table 5. In the case of α -tocopherol concentrations, high *RRR*- α -tocopherol supplement users had significantly higher concentrations (34.3 μ mol/L) than did low *all-rac*- α -tocopherol supplement users (24.9 μ mol/L). Plasma *RRR*- α -tocopherol concentrations of low and high *all-rac*- α -tocopherol supplement users (21.7 and

24.2 μ mol/L, respectively) were significantly lower than that of high *RRR*- α -tocopherol supplement users (32.1 μ mol/L). In the case of γ -tocopherol concentrations, the only significant difference was observed between nonusers (2.1 μ mol/L) and high *RRR*- α -tocopherol supplement users (0.8 μ mol/L).

Prediction of different vitamin E intake categories with the use of plasma α - and γ -tocopherol concentrations and distribution of α -tocopherol stereoisomers. With the use of ROC, plasma α - and γ -tocopherol concentrations alone showed poor ability (AUC < 0.66) to distinguish between supplement users and nonusers, between *RRR*- and *all-rac*- α -tocopherol consumers, and between *RRR*- and *all-rac*- α -tocopherol supplement users (figures not shown). Fair predictive ability (AUC = 0.72) was found between nonusers and supplement users with the use of plasma γ -tocopherol and α -tocopherol stereoisomer distribution (figure not shown). A good prediction (AUC = 0.84) was found for distinguishing between *RRR*- α -tocopherol consumers and *all-rac*- α -tocopherol supplement users with the use of plasma α -tocopherol concentration and α -tocopherol stereoisomer distribution (Figure 1A). However, for supplement users ($n = 48$) only, an excellent ROC curve (AUC = 0.91) was produced for discriminating the supplement forms (*RRR*- vs. *all-rac*- α -tocopherol) with the use of plasma α - and γ -tocopherol concentrations and α -tocopherol stereoisomer distribution, with an accuracy of 91% (Figure 1B).

Discussion

The EU RDA (14) and IOM-EAR (13) are calculated in different ways, because the IOM-EAR does not consider forms of

TABLE 3 Daily intakes of vitamin E from all sources, food sources, and supplement sources of Irish National Adult Nutrition Survey participants categorized on the basis of supplement intake level and form (synthetic or natural)¹

Vitamin E intake	Nonusers (n = 19)	Low <i>all-rac</i> - α -tocopherol supplement users (n = 19)	High <i>all-rac</i> - α -tocopherol supplement users (n = 19)	High <i>RRR</i> - α -tocopherol supplement users (n = 10)	P
All sources, mg α -tocopherol	10.3 \pm 2.8 ^c	9.8 \pm 4.0 ^c	34.6 \pm 13.1 ^b	126 \pm 150 ^a	< 0.01
Food sources, mg α -tocopherol	10.3 \pm 2.8	9.1 \pm 3.9	12.5 \pm 6.3	12.5 \pm 6.9	0.19
Supplement sources, mg α -tocopherol	—	0.7 \pm 0.7 ^c	22.1 \pm 13.2 ^b	113 \pm 147 ^a	< 0.01

¹ Values are means \pm SDs. Synthetic supplements contain *all-rac*- α -tocopherol; natural supplements contain *RRR*- α -tocopherol. Labeled means within a row without a common letter differ, $P < 0.05$.

TABLE 4 Plasma α -tocopherol stereoisomer distribution of Irish National Adult Nutrition Survey participants categorized on the basis of supplement intake level and form (synthetic or natural)¹

α -Tocopherol stereoisomer, %	Nonusers (n = 19)	Low <i>all-rac</i> - α -tocopherol supplement users (n = 19)	High <i>all-rac</i> - α -tocopherol supplement users (n = 19)	High <i>RRR</i> - α -tocopherol supplement users (n = 10)	P
<i>RRR</i> -	91.5 \pm 4.1 ^a	87.5 \pm 6.3 ^{a,b}	82.0 \pm 7.5 ^b	93.1 \pm 4.0 ^a	<0.01
<i>RRS</i> -	2.9 \pm 1.4 ^{b,c}	4.7 \pm 2.1 ^{a,b}	6.0 \pm 2.6 ^a	2.1 \pm 1.2 ^c	<0.01
<i>RSR</i> -	2.7 \pm 1.3 ^b	3.7 \pm 2.3 ^b	5.9 \pm 2.4 ^a	2.7 \pm 1.4 ^b	<0.01
<i>RSS</i> -	2.5 \pm 1.1 ^b	3.4 \pm 1.6 ^{a,b}	5.0 \pm 2.2 ^a	1.8 \pm 1.3 ^c	<0.01
2S ²	0.4 \pm 0.9	0.6 \pm 0.5	1.2 \pm 1.2	0.2 \pm 0.4	0.12

¹ Values are means \pm SDs. Synthetic supplements contain *all-rac*- α -tocopherol; natural supplements contain *RRR*- α -tocopherol. Labeled means within a row without a common letter differ, $P < 0.05$.

² Includes *SRR*-, *SRS*-, *SSR*-, and *SSS*- α -tocopherol.

tocopherol other than 2*R*- α -tocopherol forms. This can explain why >92% of the US population failed to meet the IOM-EAR (37), compared with the lower percentages observed in European studies, in which all forms of vitamin E in α -tocopherol equivalents are taken into account (38). Previous work within the cohort presented in this analysis demonstrated that the population met the recommended intake of 12 mg/d for vitamin E when all sources (food and supplements) were taken into account (39).

The EU RDA is based on all forms of vitamin E from the diet (14). However, levels of *all-rac*- α -tocopherol are not included in food composition tables (21) or routinely reported in nutritional information on food package labels and, as such, are not determined in most EU nutritional surveys (40–42). The current study, which used data from manufacturer's information or food packaging labels, estimated the *all-rac*- α -tocopherol intake. The findings indicated that the percentage of the Irish population meeting the vitamin E intake recommendation remained the same after correction with the use of an *all-rac*- to *RRR*- α -tocopherol ratio of 0.74 for 2 reasons. First, men achieved the recommended vitamin E intake from food sources only. Thus, an alteration in supplement intake would have no impact on the percentage of the population meeting the recommended intake estimation. Second, when considering the intake of women, food sources alone contributed 10.2 \pm 4.2 mg to mean daily intake, with mean daily intake from supplements being 5.6 \pm 38.5 mg, most of which was in *RRR*- form (data not shown). Hence, when *all-rac*- α -tocopherol from supplements was multiplied by 0.74, the percentage of the population meeting the recommendation did not decrease.

In considering the impact of *RRR*- and *all-rac*- α -tocopherol intake on circulating α -tocopherol stereoisomer distribution in humans, the majority of studies to date have focused on the *RRR*- α -tocopherol to *all-rac*- α -tocopherol ratio in circulation and tissues with the use of labeled α -tocopherol (11, 43, 44). However, similar to the present study, Röhrle et al. (34) found that supplemental *all-rac*- α -tocopherol in animal feed led to differ-

ences in the stereoisomer profile of bovine muscle, with an increase in *RRS*-, *RSR*-, *RSS*-, and 2*S*- α -tocopherol percentages and a decrease in *RRR*- α -tocopherol percentage. In a human study, Kiyose et al. (9) showed that after administration of 300 mg/d *all-rac*- α -tocopherol acetate for 28 d, the percentage of 2*R*-stereoisomers in both LDL and HDL significantly decreased, but the percentage of 2*R*-stereoisomers was still significantly higher than that of 2*S*-stereoisomers. These changes in lipoproteins were similar to the present findings in plasma; however, the percentage of *RRR*-, *RRS*-, *RSR*-, and *RSS*- α -tocopherol was not measured by Kiyose et al.

As with earlier studies (45, 46), our results showed that high *RRR*- α -tocopherol intake led to a significant increase in plasma α -tocopherol concentration and a decrease in plasma γ -tocopherol concentration. It is known that α -tocopherol supplementation may reduce plasma γ -tocopherol concentration because of competition for hepatic transfer, which preferentially salvages α -tocopherol from catabolism, promoting its incorporation into nascent VLDL and circulation (47). On the other hand, γ -tocopherol is rapidly metabolized to 2,7,8-trimethyl-2-(β -carboxyethyl)-6-hydroxychroman and excreted in the urine (48). Chopra et al. showed that both *RRR*- and *all-rac*- α -tocopherol significantly suppressed plasma γ -tocopherol concentration to the same extent; this may be attributed to the higher level of supplementation 800 mg/d compared with the 0.7–113 mg/d in the current study (49). Some studies have measured plasma α -tocopherol concentration after oral administration of *RRR*- or *all-rac*- α -tocopherol, but the individual α -tocopherol stereoisomer distribution and concentration have not been reported in nutritional surveys (49, 50). The uniqueness of the present study lies in the assessment of the effect of habitual intake of *RRR*- and *all-rac*- α -tocopherol from supplements on plasma *RRR*- α -tocopherol concentration.

Consumption of *all-rac*- α -tocopherol from supplements increased total vitamin E intake, while at the same time led to a significant reduction in plasma *RRR*- α -tocopherol concentration.

TABLE 5 Plasma α -tocopherol, *RRR*- α -tocopherol, and γ -tocopherol concentrations of Irish National Adult Nutrition Survey participants categorized on the basis of supplement intake level and form (synthetic or natural)¹

Plasma concentration, μ mol/L	Nonusers (n = 19)	Low <i>all-rac</i> - α -tocopherol supplement users (n = 19)	High <i>all-rac</i> - α -tocopherol supplement users (n = 19)	High <i>RRR</i> - α -tocopherol supplement users (n = 10)	P
α -tocopherol	30.1 \pm 8.1 ^{a,b}	24.9 \pm 4.7 ^b	29.8 \pm 8.0 ^{a,b}	34.3 \pm 6.9 ^a	0.01
<i>RRR</i> - α -tocopherol ²	27.5 \pm 7.3 ^{a,b}	21.7 \pm 3.8 ^b	24.2 \pm 6.1 ^b	32.1 \pm 7.2 ^a	<0.01
γ -tocopherol	2.1 \pm 1.5 ^a	1.6 \pm 0.8 ^{a,b}	1.4 \pm 0.7 ^{a,b}	0.8 \pm 0.6 ^b	0.04

¹ Values are means \pm SDs. Synthetic supplements contain *all-rac*- α -tocopherol; natural supplements contain *RRR*- α -tocopherol. Means within a row without a common letter differ, $P < 0.05$.

² Derived from the plasma α -tocopherol concentrations from Table 5 and the *RRR*- α -tocopherol distribution from Table 4.

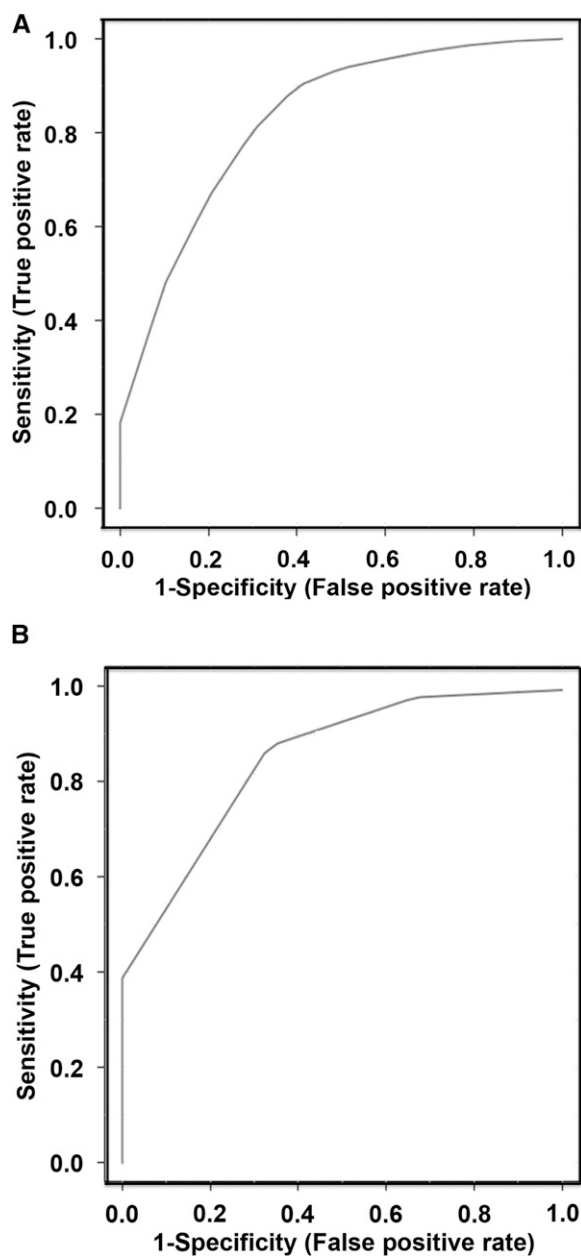


FIGURE 1 Receiver operating characteristic curves produced to discriminate between *RRR*- α -tocopherol consumers ($n = 29$; 19 nonusers and 10 high *RRR*- α -tocopherol supplement users) and *all-rac*- α -tocopherol supplement users ($n = 38$; 19 low and 19 high *all-rac*- α -tocopherol supplement users) (A) and between *RRR*- α -tocopherol supplement users (10 high *RRR*- α -tocopherol supplement users) and *all-rac*- α -tocopherol supplement users ($n = 38$; 19 low and 19 high *all-rac*- α -tocopherol supplement users) (B). Plasma α -tocopherol concentrations and distribution of α -tocopherol stereoisomers to discriminate between *RRR*- α -tocopherol consumers (nonusers and high *RRR*- α -tocopherol supplement users) and *all-rac*- α -tocopherol supplement users (low and high *all-rac*- α -tocopherol supplement users), $n = 67$; AUC = 0.84 (95% CI: 0.71, 0.96) (A). Plasma α - and γ -tocopherol concentrations and distribution of α -tocopherol stereoisomers to discriminate between *RRR*- α -tocopherol supplement users (high *RRR*- α -tocopherol supplement users) and *all-rac*- α -tocopherol supplement users (low and high *all-rac*- α -tocopherol supplement users), $n = 48$; AUC = 0.91 (95% CI: 0.72, 1.0) (B).

It has been established that after consumption of *all-rac*- α -tocopherol, *2R*- α -tocopherols are well retained in circulation;

however, *2S*- α -tocopherols are actively metabolized and eliminated in humans (51). Therefore, it may be important to understand potential differing impacts of *RRR*- and *all-rac*- α -tocopherol intake on health. To date, most European nutrition surveys (52), e.g., the UK National Diet and Nutrition Survey (53) and the French the SU.VI.MAX (Supplémentation en Vitamines et Minéraux Antioxydants) study (41), have considered overall vitamin E intake and its effect on plasma α - and γ -tocopherol concentrations, whereas the US NHANES (54, 55) assessed only *2R*- α -tocopherol intake. In large epidemiologic studies, which have demonstrated a link between vitamin E intake and plasma α -tocopherol concentration and biomarkers of cardiovascular disease risk (56–58), there was no distinction made between intake of *RRR*- and *all-rac*- α -tocopherol, and, as such, any associated health impact of *RRR*- and *all-rac*- α -tocopherol intake remains unknown.

Within the current study, with the use of ROC analysis, it was clear that plasma measurements did not permit discrimination between supplement users and nonusers. However, a reasonable prediction was found in classifying an individual as a consumer of *all-rac*- α -tocopherol or not. A good predictive ability to discriminate between *RRR*- and *all-rac*- α -tocopherol supplement users with optimal sensitivity and specificity was obtained. The identification of plasma measurements related to supplement intake forms could improve the assessment of the relation of *RRR*- and *all-rac*- α -tocopherol intake with disease. These prediction methods would allow researchers of large epidemiologic studies to further analyze existing plasma samples and retrospectively assign individuals as *RRR*- or *all-rac*- α -tocopherol consumers. Additionally, if an individual was known to be a supplement user, the predictors could distinguish between *RRR*- α -tocopherol and *all-rac*- α -tocopherol supplement intake, which in turn would allow investigations into the potential effect of *RRR*- and *all-rac*- α -tocopherol intake on health.

Although the present study provides strong evidence for the link between plasma α -tocopherol stereoisomer distribution and the form of α -tocopherol intake from supplements, there are limitations. First, the number of participants was small and future studies with larger cohorts will be necessary to validate the current findings. Second, the *all-rac*- α -tocopherol intake of high *all-rac*- α -tocopherol supplement users was relatively low compared with the *RRR*- α -tocopherol intake of high *RRR*- α -tocopherol supplement users; thus, participants with a higher intake of *all-rac*- α -tocopherol should be selected in future studies. Third, supplement users in this study consumed supplements from single sources (*RRR*- or *all-rac*- α -tocopherol), as opposed to combinations thereof. Finally, the present work cannot be extrapolated to those countries that only take *2R*- α -tocopherol intake into account when determining vitamin E recommendations (59), and is limited to regions of the world that consider all forms of vitamin E to be α -tocopherol equivalents in estimating vitamin E intake and recommended levels, e.g., the European Union (14), Australia (60), and the United Kingdom (61).

Nevertheless, the study illustrates the influence of *RRR*- and *all-rac*- α -tocopherol intake from supplements on the percentage of Irish adults meeting the vitamin E recommendation, plasma α - and γ -tocopherol concentrations, and distribution of α -tocopherol stereoisomers. Importantly, it has shown that plasma α - and γ -tocopherol concentrations and distribution of α -tocopherol stereoisomers can be used to discriminate between *RRR*- and *all-rac*- α -tocopherol supplement users. Further work is required toward translation of the present findings to improve our understanding of the potential varied impacts of *RRR*- and *all-rac*- α -tocopherol intake on the risk of diseases.

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YZ and MJG designed the research; FJM, MJG, and ERG conducted the research; YZ, BAM, and LB analyzed the data; and YZ, FJM, and ERG wrote the paper. All authors read and approved the final manuscript.

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