British Journal of Nutrition (2006), **95**, 477–487
DOI: 10.1079/BJN20051667

Bioavailability of α-tocopherol stereoisomers in rats depends on dietary doses of *all-rac*- or RRR-α-tocopheryl acetate

Søren K. Jensen*, Jan V. Nørgaard and Charlotte Lauridsen

Department of Animal Health, Welfare and Nutrition, Danish Institute of Agricultural Sciences, Research Centre Foulum, Box 50, DK-8830 Tjele, Denmark

(Received 31 August 2005 – Revised 2 November 2005 – Accepted 8 November 2005)

The biological function of the stereoisomers of α-tocopherol is believed to depend on their bioavailability. Assessment of bioavailability within the body is therefore considered to be a good and easy way to predict biological value. The separation of α-tocopherol methyl ethers by chiral column HPLC is a good and easy tool with which to study the distribution of α-tocopherol stereoisomers. The objective of this investigation was to evaluate the bioavailability and distribution of the stereoisomers of α-tocopherol in the plasma and tissue in growing rats fed 25, 50, 100 or 200 mg/kg diet of either RRR- or *all-rac*-α-tocopheryl acetate for 10 d. The ratio between the two vitamin E sources based on their α-tocopherol concentration in plasma and tissues varied in the plasma between 1·04 and 1·74 and in tissues, ratios of 0·84–1·24 for liver, 0·34–1·59 for lung and 0·75–1·50 for spleen were obtained. An increasing dietary level of *all-rac*-α-tocopheryl acetate decreased the proportion of RRR-α-tocopherol, whereas the other stereoisomers were not affected. RRS-α-Tocopherol was present in the highest proportion, followed by RSR-, RSS- and RRR-α-tocopherol. In contrast to the other tissues and plasma, the liver contained the highest proportion (29–33 %) of the four 2S stereoisomers of total α-tocopherol. Rats fed RRR-α-tocopheryl acetate for 10 d showed a significant increase in the plasma and tissue content of RRR-α-tocopherol and a simultaneous decrease in the other three 2R isomers, whereas the absolute content of the 2S isomers was unaffected. In adipose tissue, concentrations of the three synthetic 2R isomers remained constant, whereas there was a steep increase in the content of RRR-α-tocopherol.

Bioavailability: Biodiscrimination: Absorption: α-Tocopherol stereoisomers: Rats: Vitamin E

All natural occurring tocopherols are optically active and have the same stereochemical configuration in their side chain. In nature, eight tocopherols with vitamin E activity are found, these being designated α-, β-, γ- and δ-tocopherol and their corresponding tocotrienols. α-Tocopherol, also termed RRR-α-tocopherol (2,5,7,8-tetramethyl-2R-(4′R,8′R,12-trimethyltridecyl)-6-chromanol)), has the highest biological activity and is maintained at the highest level in the plasma and tissues of animals and human subjects (Kayden & Traber, 1993). However, the majority of the vitamin E used to supplement food and feed is of synthetic origin and is designated *all-rac*-α-tocopheryl acetate (2,5,7,8-tetramethyl-2RS-(4′RS,8′RS,12-trimethyltridecyl)-6-chromanol). *All-rac*-α-Tocopherol is an equimolar mixture of all eight possible stereoisomers and is designated RRR, RRS, RSS, RSR, SRR, SSR, SRS and SSS; thus, only the RRR stereoisomer possesses the natural configuration.

The official biopotency factors of 1·00 for *all-rac*-α-tocopheryl acetate and 1·36 for RRR-α-tocopheryl acetate (United States Pharmacopeial Convention, 1979) are based mainly on results from the rat resorption–gestation test, which was first published by Harris & Ludwig (1949). Weiser & Vecchi (1982) studied the relative biopotencies of all eight stereoisomers of α-tocopherol by the rat resorption–gestation test and showed that α-tocopherol with a 2R configuration had a higher biopotency factor value than α-tocopherol with the 2S configuration.

The biological function of vitamin E is assumed to reflect the concentration of the vitamin E in the given tissue. This relationship is reflected in bioassays such as for haemolysis, curative myopathy, muscle dystrophy and other assays concerning the tissue availability of α-tocopherol (Scherf *et al.* 1996).

Chromatographic techniques based on chiral separation allow direct measurement of the individual stereoisomers in fluids and tissues (Weiser *et al.* 1996), and provide an important tool as a large number of samples can be analysed in order to, for example, make direct comparisons of the bioavailability found in rats with that in other animals. In studies with rats, a good consistency between the classical resorption–gestation test and the bioavailability of the individual stereoisomers in fluids and tissues has been shown (Scherf *et al.* 1996; Weiser *et al.* 1996). For practical and ethical reasons, the resorption–gestation test is not applicable to larger animals or human subjects. Furthermore, in most practical applications, the animals of interest are not vitamin E deficient, and it will often be more relevant to compare different vitamin E forms at normal physiological levels.

Few experiments have included the use of different dosages of the compared vitamin preparations within physiologically

* **Corresponding author:** Dr S. K. Jensen, fax +45 89991166, email sorenKrogh.Jensen@agrsci.dk

relevant dosages. As α-tocopherol stereoisomers are treated and metabolized in the body in different ways, it is not likely that one single ratio will be able to explain the difference, because the synthetic and the natural vitamin E are not equivalent in any dosage ratio (Blatt *et al.* 2004).

Other methods dealing with the bioavailability and metabolism of tocopherols are ^{2}H-labelled α-tocopherol in conjunction with GC–MS (Ingold *et al.* 1987) or HPLC–MS (Lauridsen *et al.* 2001). These methods allow newly absorbed vitamin E to be distinguished from the unlabelled vitamin E already present in the body (Burton *et al.* 1998). In addition, the ^{2}H-vitamin E technique allows a direct comparison of two distinctly labelled vitamin E forms in the same animal, whereby most of the variation attributable to differences between individuals, and factors that change with time, can be eliminated (Acuff *et al.* 1994). One disadvantage of this technique is that *all-rac*-α-tocopherol is a mixture of eight stereoisomers, but the mass spectrometer does not detect the chiralities of the carbons at positions 2′, 4′ and 8′ in the tocopherol molecule.

The purpose of the present experiment was to investigate the effect of different dietary levels of RRR-α-tocopheryl acetate or *all-rac*-α-tocopheryl acetate on total α-tocopherol concentration in the plasma, tissues and faeces, and the distribution of the individual stereoisomers in plasma and tissue as a function of different dietary doses.

Materials and methods

Animals and diets

A total of forty-five male Wistar rats were supplied from Taconic Farms, Inc. (Acquires M&B A/S; Ry, Denmark). The rats were obtained at 4 weeks old and weighed 76 (SD 3) g. They were housed individually in metabolism cages at the Danish Institute of Agricultural Sciences (Tjele, Denmark). The protocol concerning animal experimentation and the care of experimental animals used in this experiment complied with the Danish Animal Experiments Inspectorate, Ministry of Justice, Copenhagen.

The composition of the experimental diets is shown in Table 1. The basal diet was semi-purified and without detectable vitamin E. The diets contained increasing amounts of α-tocopherol (25, 50, 100, 200 mg/kg feed) added as *all-rac*-α-tocopheryl acetate (groups S25, S50, S100, S200) or as RRR-α-tocopheryl acetate (groups N25, N50, N100, N200). RRR-α-Tocopheryl acetate was provided by Pharmalett A/S (Kolding, Denmark), and *all-rac*-α-tocopheryl acetate was provided by DSM Nutritional Products A/S (Brøndby, Denmark).

Table 1. Composition of basal diet and analysed content of α-tocopherol in experimental diets

Ingredient	g/kg	
Maize starch	701	
Casein	98	
Sugar	76	
Soyabean oil	44	
Cellulose	44	
Vitamin and mineral mix*	37	
Group	α-tocopherol (mg/kg diet) (*n* 2)	
	Mean	SD
All-rac-α-tocopheryl acetate		
Basal diet	1·6	0·2
S25	23	1·4
S50	54	4·2
S100	108	3·5
S200	219	0·7
RRR-α-tocopheryl acetate		
N25	28	2·8
N50	62	9·2
N100	106	2·1
N200	211	4·9

*The vitamin and mineral mix provided per kilogram diet: retinol (retinyl acetate), 3500 μg; menadione, 5·7 mg; $CaCl_2$, 970 mg; folic acid, 970 μg; nicotin amide, 17·4 mg; calcium pantothenate, 7 mg; riboflavin, 2800 μg; thiamine hydrochloride, 3500 μg; pyridoxine chloride, 5500 μg; cyanocobalamine, 42 μg; biotin, 350 μg; Vitamin D_3, 900 μg; $Ca(C_6H_5O_7)_2$ $4H_2O$, 12·29 g; KH_2PO_4, 8·71 g; KCl, 4·97 g; $CaHPO_4$ $2H_2O$, 4·50 g; NaCl, 3·07 g; $CaCO_3$, 2·71 g; $MgSO_4$, 1·53 g; $(MgCO_3)_4$ $Mg(OH)_2$ $5H_2O$, 1·40; $ZnSO_4$ $7H_2O$, 438 mg; ammonium ferricitrate, 305 mg; $CuSO_4$ $5H_2O$, 40 mg; NaF, 20 mg; $MnSO_4$ $5H_2O$, 8000 μg; $Al_2(SO_4)_2SO_4$ $24H_2O$, 3600 μg, KI, 1600 μg. These amounts were premixed with maize starch, and the vitamins and minerals were provided by Merck (Damstadt, Germany) and Bie and Berntsen (Aabyhoej, Denmark).

Protocol

During the initial 2 d of the experiment, all rats were fed a basal vitamin-E-free diet. On day 2, five rats were killed in order to make an initial reference value with respect to α-tocopherol status and composition. The remaining rats were randomly distributed between the eight experimental diets. Throughout the experimental period, the rats were fed a daily amount of 10 g DM with free access to fresh drinking water. The rats were weighed on days 2, 5, 9 and 12. The amount of feed consumed was determined during three periods at days 2–4, 5–9 and 10–12. To determine the apparent absorption of α-tocopherol, faeces were collected each day from day 5 to day 9 and stored at −20 °C. On day 12, all rats were deprived of feed and were selected at random before being killed individually in a CO_2 atmosphere.

Blood samples (approximately 3 ml) were obtained by heart puncture of the killed rats into 4 ml sodium-heparinized tubes and stored on ice until centrifugation at 3000 ***g*** to obtain plasma. Liver, lung and spleen were collected and weighed, and were, together with the white adipose tissue (retroperitoneal) and plasma obtained, stored at −20 °C until analysis.

Tocopherol analyses

The quantitative determination of α-tocopherol was performed by HPLC after saponification and extraction into heptane as outlined by Jensen *et al.* (1999). In brief, tissue was homogenized in twice the amount of ethanol by an Ultra-Turrax homogenizer (IKA–Werke GmbH, Staufen, Germany), while being kept on ice. Aliquots of the homogenates corresponding to 150 mg spleen, 200 mg lung and 167 mg liver were saponified in a mixture of ethanol, methanol, ascorbic acid (20 % w/v) and KOH–water (1:1 w/v) at 80 °C for 30 min, subsequently cooled and extracted into two portions of 5 ml heptane. Adipose tissue (100 mg) was dissolved in 10 ml heptane and solubilized at 80 °C for 2 h prior to injecting 100 mg into the HPLC. Feed (1·000 g), faeces (1·000 g) and plasma (0·300 ml) were saponified, and either 50 μl or 100 μl were injected into the HPLC.

The HPLC column to determine tocopherol consisted of a 4·0 × 125 mm HS-5-Silica column (Perkin-Elmer GmbH, Überlingen, Germany). The mobile phase consisted of heptane containing 2-propanol (3·0 ml/l) and degassed with He. The flow rate was 3·0 ml/min. A comparison of retention time and peak areas with Merck (Damstadt, Germany) external standards was used to obtain the identification and quantification of the tocopherol. Fluorescence detection was performed with an excitation wavelength of 290 nm and an emission wavelength of 327 nm.

Stereoisomers of α-tocopherol were analysed by HPLC as follows. The remaining heptane extract was evaporated to exact dryness under a stream of N_2 gas. The α-tocopherol was then derivatized to its methyl ether following the method described by Drotleff & Ternes (2001). The methyl ether derivative was extracted with 1·50 ml heptane, of which 100 μl was injected into the HPLC column. Chromatographic separation was achieved on a Chiralcel OD-H column (25 × 0·46 cm, 5 μm particle size, cellulose tris (3,5-dimethylphenylcarbamate; Daicel Chemical Industries, Ltd, Tokyo, Japan) with heptane modified with 0·0065 % 2-propanol. This method allows the separation of the eight stereoisomers of α-tocopherol into five peaks (Fig. 1): the first peak contains the four 2SR/SR/S forms, the second peak contain the 2RSS-α-tocopherol, the third peak contains 2RRS-α-tocopherol, the fourth peak contains 2RRR-α-tocopherol, and the fifth peak contains 2RSR-α-tocopherol.

Statistics and mathematical calculations

For all statistical models, there was a replacement of 'dose' (25, 50, 100, 200 mg/kg) as an explaining variable with a variable 'exact-dose' (from feed analyses) and a variable 'dose-consumed' (calculated by the 'exact-dose' multiplied by the amount of feed consumed in the experimental period from day 2–12). 'Dose' and 'exact-dose' were treated as fixed-effect variables, and 'dose-consumed' as a random-effect variable. No models changed outcome by changing variable. Thus, the variable 'dose' was used in all models described below.

The overall statistical model, which was used to analyse differences in vitamin E concentrations in the liver, lung, spleen and adipose tissue, was:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_{(k)} + \delta_{(l)} + \eta_{(m)} + (\alpha\beta)_{ij} + \varepsilon_{ijklm} \quad (1)$$

where Y_{ijklm} is the responding vitamin E concentration (μg/g) of either liver, lung, spleen or adipose tissue, μ is the overall mean, α_i is the fixed effect of treatment (*all-rac-* or RRR-α-tocopheryl acetate), β_j is the fixed effect of dose (25, 50, 100, 200 mg/kg), $\gamma_{(k)}$ is the co-variate organ weight, $\delta_{(l)}$ is the co-variate gain (weight increment of the rat), $\eta_{(m)}$ is the co-variate of feed consumed by the rat from day 2 to day 12, $(\alpha\beta)_{ij}$ is the interaction between vitamin E and dose, and ε_{ijklm} is the random error assuming that $\{\varepsilon_{ijklm}\}$ is approximately equal to $N(0,\sigma^2)$. Plasma concentrations (μg/ml) were analysed by using equation (1) but omitting the factor 'organ weight'. ANOVA was used to compare differences between stereoisomers within the same treatment and differences within each stereoisomer as a function of different dosages.

The apparent absorption coefficients for α-tocopherol were calculated according to the equation:

$$(\text{vitamin E}_{(\text{consumed})} - \text{vitamin E}_{(\text{excreted})}) \times 100/\text{vitamin E}_{(\text{consumed})} \quad (2)$$

and values were provided through a collection of faeces on days 5–12, a registration of feed consumption on days 5–12 and a feed analysis of the exact content of α-tocopherol. Statistical analysis of the apparent absorption coefficients was performed by conducting a model similar to that in equation 1, omitting the variables of organ weight and gain.

The relative bioavailability of each stereoisomer in plasma and tissues was calculated according to the following two equations:

$$2\text{R-}\alpha\text{-tocopherols} = [\text{percentage of stereoisomer}] \times 8/100 \quad (3)$$

$$2\text{S-}\alpha\text{-tocopherols} = [\text{percentage of stereoisomer}] \times 2/100 \quad (4)$$

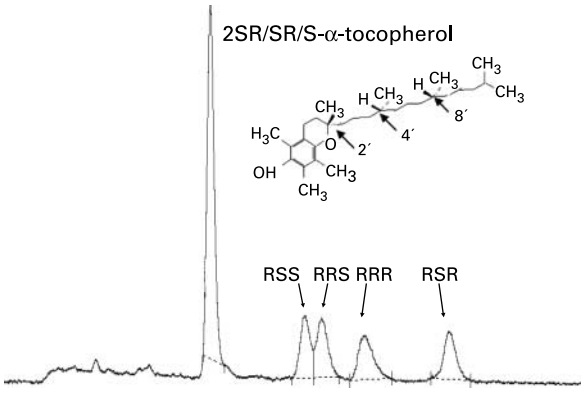

Fig. 1. HPLC chromatogram of methyl ethers of *all-rac*-α-tocopherol separated on a Chiralcel OD-H column. The arrows on the molecule shows the chiral centres at position 2′, 4′ and 8′, respectively. 2SR/SR/S denotes the mixture SSS-, SRR-, SSR-, SRS-α-tocopherol; RSS, RRS, RRR and RSR denote RSS-, RRS-, RRR- and RSR-α-tocopherol, respectively. UFO, xxxxx.

Results

Animal performance was similar for all groups resulting in an overall weight increase of 27·7 ± 3·0 g. No diseased animals were observed. The weight of liver, lung and spleen of the five rats killed at the beginning of the experiment averaged 3·00 (0·25), 0·588 (0·06) and 0·358 (0·05) g, respectively. At the end of the experiment, the organ weights averaged 3·75 (0·33), 0·807 (0·13) and 0·283 (0·03) g for liver, lung and spleen, respectively (*n* 40).

Apparent absorption coefficient

The apparent absorption coefficient of DM did not differ between the groups. The average apparent absorption coefficient was higher for RRR-α-tocopherol than for *all-rac*-α-tocopherol. RRR-α-Tocopherol was the only stereoisomer

found in faeces from the groups N25–N200, whereas the proportion of 2S isomers increased in the faeces with increasing concentration of *all-rac*-α-tocopheryl acetate in the feed ($P<0·001$; Table 2).

α -Tocopherol concentrations in plasma and tissues

At the beginning of the experiment, the highest content of α-tocopherol was found in the spleen, followed by adipose tissue, lung, liver and plasma (Table 3). The highest concentration of the four 2S stereoisomers was found in liver and the lowest concentration in plasma. α-Tocopherol in the plasma increased with increasing dietary level of both sources of α-tocopherol ($P<0·001$). At 25, 50 and 100 mg dietary inclusion, the natural source gave the highest concentration, whereas groups S200 and N200 showed equal plasma concentrations (Table 4). This caused an interaction between vitamin source and dose ($P=0·012$), and the ratio between the two forms of α-tocopherol decreased from 1·74 at 25 mg/kg feed to 1·04 at 200 mg/kg feed. α-Tocopherol in liver increased with increasing dose ($P<0·001$), but no difference between the two vitamin sources was found ($P=0·59$).

In lung tissue, the difference between the vitamin sources was pronounced. Rats from group S25 and group N200 had a significantly lower concentration of α-tocopherol than rats from all the other groups ($P<0·05$), but there was no difference between rats from groups S25 and N200. Thus, the ratio between RRR and *all-rac*-α-tocopherol decreased from 1·59 for the 25 mg/kg groups to 1·09 and 0·90 for the 50 and 100 mg/kg diet and to 0·34 for the 200 mg/kg diet group.

Spleen showed a similar pattern to that observed for lung tissue, that is, the rats fed *all-rac*-α-tocopheryl acetate

Table 2. Apparent absorption coefficients of α-tocopherol and DM, and relative distribution of α-tocopherol stereoisomers in feed and faeces

(Means and standard deviations)

		Apparent absorption coefficient*										
		25		50		100		200		Mean		
Tocopherol source		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P_{dose}
RRR-		86·8	3·9	87·5	3·0	86·9	3·7	87·3	1·8	87·6	2·6	0·97
All-rac-		77·2^{b}	2·9	85·4^{a}	3·0	83·9^{a}	2·0	83·3^{a}	3·0	82·4	4·3	0·005
P_{source}		<0·0001		0·27		0·12		0·04		<0·0001		
DM digestibility		95·8		95·8		95·6		95·7		95·7		0·76
		Relative distribution of α-tocopherol stereoisomers										
	Group	RRR-		RRS-		RSR-		RSS-		Σ2R		Σ2S	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Feed†		12·5	0·4	11·8	0·5	13·4	1·0	13·2	0·4	50·9	0·4	49·1	0·4
Faeces	S25	13·3^{a}	0·7	14·7^{a}	0·7	13·1^{a,b}	1·3	12·2	0·5	53·3^{a}	1·5	46·7^{b}	1·5
	S50	13·0^{a,b}	0·5	13·5^{b}	0·5	13·8^{a}	0·7	12·1	0·5	52·4^{a}	0·9	47·6^{b}	0·9
	S100	12·8^{a,b}	0·4	13·2^{b}	0·3	12·8^{b}	0·3	12·0	0·4	50·8^{b}	0·6	49·2^{a}	0·6
	S200	12·5^{b}	0·1	13·1^{b}	0·3	13·1^{a,b}	0·2	12·0	0·1	50·7^{b}	0·2	49·3^{a}	0·4
	P_{dose}	0·09		0·0002		0·22		0·84		0·0007		0·0007	
Feed‡										100			
Faeces	N25	100		0		0		0		100		0	
	N50	100		0		0		0		100		0	
	N100	100		0		0		0		100		0	
	N200	100		0		0		0		100		0	

^{a,b} Mean values within a row with unlike superscript letters were significantly different ($P<0·05$). Dose × source, $P=0·04$.
* Apparent absorption coefficients are percentages (equation (2) in Materials and Methods) for the doses 25–200 mg/kg diet.
† Average of replicate analysis of S25, S50, S100, S200; *n* 8.
‡ Average of replicate analysis of N25, N50, N100, N200; *n* 8.

Table 3. Concentration and composition of α-tocopherol stereoisomers in plasma and tissues of the rats at the beginning of the experiment

	α-Tocopherol (nmol/g tissue)*		α-Tocopherol stereoisomers, relative composition (%)					
	Mean	SD	RRR-	RRS-	RSR-	RSS-	Σ2S	*P*
Plasma	14^{c}	2	26·1^{A}	25·8^{Aa}	21·3^{AB}	21·4^{B}	5·4^{Cc}	<0·001
Liver	16^{c}	3	23·6^{A}	19·8^{Bb}	18·5^{C}	21·7^{B}	16·3^{BCa}	<0·001
Lung	30^{b}	5	27·7^{A}	20·8^{Bab}	22·7^{AB}	18·6^{B}	10·3^{Cb}	<0·001
Spleen	45^{a}	7	26·9^{A}	23·8^{Ba}	20·5^{C}	20·2^{C}	8·6^{Db}	<0·001
Adipose tissue	43^{a}	8	25·9^{A}	23·6^{Ba}	20·6^{C}	20·0^{C}	9·8^{Db}	<0·001
P	<0·001		0·14	<0·001	0·19	0·30	<0·001	

* Value in plasma: nmol/ml plasma.
^{a,b,c} Mean values within a column with unlike lower case letters were significantly different ($P<0·05$).
^{A,B,C} Mean values within a row with unlike capital letters were significantly different ($P<0·05$).

Table 4. Plasma and tissue (wet weight) concentration of α-tocopherol in plasma and tissues from rats fed RRR- or *all-rac*-α-tocopheryl acetate

(Means and standard deviations, *n* 5)

	α-Tocopherol (mg/kg diet)										
	25		50		100		200		Mean		Effect of dosage
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*
Plasma (μmol/l)											
RRR	13[b]	2	15[b]	1	20[a]	4	19[a]	3	17	4	0·0006
All-rac	7[c]	1	12[b]	2	15[a]	4	18[a]	2	13	5	<0·0001
Ratio*	1·74		1·28		1·29		1·04		1·26		
Effect of source *P*	<0·0001		0·02		0·02		0·74		<0·0001		
Liver (nmol/g)											
RRR-	6[d]	2	8[c]	2	13[b]	2	17[a]	2	11	5	<0·0001
All-rac-	6[c]	2	10[b,c]	3	13[a,b]	4	14[a]	3	11	4	0·002
Ratio*	0·96		0·84		1·01		1·24		1·04		
Effect of source *P*	0·86		0·30		0·95		0·04		0·59		
Lung (nmol/g)											
RRR-	40[a]	8	51[a]	5	50[a]	16	21[b]	5	40	16	0·0005
All-rac-	25[b]	9	47[a]	7	56[a]	6	60[a]	20	47	18	0·001
Ratio*	1·59		1·09		0·90		0·34		0·86		D × S†
Effect of source *P*	0·04		0·55		0·44		<0·0001		0·06		< 0·0001
Spleen (nmol/g)											
RRR-	49	8	54	5	63	24	61	23	57	17	0·57
All-rac-	33[c]	9	49[b]	10	63[b]	15	82[a]	12	57	21	<0·0001
Ratio*	1·50		1·10		1·00		0·75		1·01		
Effect of source *P*	0·09		0·60		0·99		0·04		0·94		
Adipose tissue (nmol/g)											
RRR-	49	10	79	37	74	29	89	31	73	30	0·19
All-rac-	53	12	54	9	49	21	56	13	53	13	0·86
Ratio*	0·92		1·47		1·51		1·58		1·37		
Effect of source *P*	0·76		0·09		0·09		0·03		0·01		

[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* The ratio is calculated by dividing the concentration of α-tocopherol from the RRR-fed groups by the α-tocopherol concentration from the *all-rac*-fed groups.
† D × S = interaction between dose and source.

showed increasing concentrations with increasing content in the feed ($P<0·001$), whereas the rats fed RRR already had values close to the plateau with the 25 mg/kg diet. Therefore, no effect of dose was seen in the N groups ($P=0·57$). Like lung tissue, the ratio in spleen between RRR and *all-rac*-α-tocopherol decreased with increasing dosage.

Rats fed the N diets deposited more α-tocopherol in their adipose tissue than did rats fed the S diets ($P=0·01$). Thus, rats from group N200 deposited 1·37 times more α-tocopherol than rats from group S200 ($P=0·03$).

Distribution of stereoisomers of α-tocopherol in rats fed all-rac-α-tocopheryl acetate

The relative distribution of stereoisomers of α-tocopherol in plasma and tissues from rats fed the *all-rac*-α-tocopheryl acetate diets is shown in Table 5. With increasing dietary content of *all-rac*-α-tocopheryl acetate in the feed, the proportion of RRR-α-tocopherol decreased in plasma ($P<0·001$), liver ($P<0·001$), lung ($P=0·01$) and adipose tissue ($P=0·002$). The three synthetic 2R isomers remained fairly constant within each of the tissues, and only small changes were observed. RRS-α-tocopherol constituted the highest proportion of the four 2R stereoisomers in plasma and all measured tissues with the exception of the liver, where the 2S stereoisomers constituted the highest proportion. The four 2S stereoisomers decreased in the lung ($P=0·006$) and the spleen ($P=0·03$) with increasing content of *all-rac*-α-tocopheryl acetate in the feed. The lowest proportion of 2S-α-tocopherols was found in the plasma (Table 5).

Relative bioavailability of the different stereoisomers based on their abundance in plasma and tissue

The relative bioavailability of each stereoisomer compared with *all-rac*-α-tocopherol in plasma and tissues as a function of different dietary inclusion levels is shown in Fig. 2. The bioavailability of each stereoisomer differed between plasma and tissues and varied with dosage, especially for RRR-α-tocopherol. All 2R stereoisomers had relative bioavailabilities greater than 1, whereas the 2S stereoisomers in all cases had bioavailabilities lower than 1.

The bioavailability of RRR-α-tocopherol was highest in plasma, lowest in liver and decreased clearly with increasing levels of *all-rac*-α-tocopheryl acetate in the feed. In general, the bioavailability of the three synthetic 2R stereoisomers was higher than that of RRR-α-tocopherol.

For the 2S stereoisomers, the lowest bioavailability was found in plasma (0·18), whereas spleen and lungs showed bioavailabilities of about 0·27. The highest bioavailability was found in liver and averaged 0·61. Overall, RRR-α-tocopherol

Table 5. Relative proportion of α-tocopherol stereoisomers in plasma and tissues of rats fed variable doses of *all-rac*-α-tocopheryl acetate

		α-Tocopherol stereoisomers (%)*					
	Group	RRR-	RRS-	RSR-	RSS-	Σ2S	*P*
Plasma	S25	22·3^{A,B,a,b}	24·1^{A}	24·2^{A}	19·9^{B}	9·5^{C}	<0·0001
	S50	23·7^{A,a}	24·9^{A}	22·5^{A}	20·9^{B}	8·0^{C}	<0·0001
	S100	21·7^{B,C,b}	25·5^{A}	23·4^{A,B}	20·9^{C}	8·5^{D}	<0·0001
	S200	18·2^{D,c}	26·5^{A}	23·6^{B}	20·8^{C}	10·8^{E}	<0·0001
	Effect of dose *P*	<0·0001	0·08	0·56	0·27	0·17	
Liver	S25	19·7^{B,a}	17·5^{C}	17·5^{C}	16·0^{C}	29·2^{A}	<0·0001
	S50	17·9^{B,b}	17·9^{B}	17·7^{B}	17·4^{B}	29·1^{A}	<0·0001
	S100	16·3^{B,c}	17·7^{B}	17·8^{B}	16·9^{B}	31·2^{A}	<0·0001
	S200	13·3^{C,d}	17·5^{B}	18·6^{B}	17·4^{B}	33·1^{A}	<0·0001
	Effect of dose *P*	<0·0001	0·92	0·47	0·33	0·38	
Lung	S25	21·0^{A,a}	20·8^{cA,B}	20·5^{A,B}	21·6^{A,b}	16·1^{B,a}	<0·0001
	S50	20·8^{B,a}	24·4^{a,A}	20·8^{B}	20·5^{B,b}	13·5^{C,a,b}	<0·0001
	S100	21·1^{B,a}	25·2^{a,A}	21·6^{B}	21·3^{B,b}	10·9^{C,b}	<0·0001
	S200	18·6^{C,b}	22·9^{b,B}	21·6^{B}	24·9^{A,a}	12·0^{D,b}	<0·0001
	Effect of dose *P*	0·01	<0·0001	0·71	<0·0001	0·006	
Spleen	S25	17·8^{B,C}	24·2^{A}	18·6^{c,C}	21·6^{A,B}	17·8^{a,D}	<0·0001
	S50	19·5^{A,B,C}	23·6^{A}	0·4^{b,c,A,B}	22·3^{B}	14·1^{a,b,C}	<0·0001
	S100	21·0^{B}	24·1^{A}	20·8^{b,B}	22·1^{A,B}	12·0^{b,C}	<0·0001
	S200	16·7^{D}	25·7^{A}	23·0^{a,B}	21·8^{C}	12·8^{b,E}	<0·0001
	Effect of dose *P*	0·13	0·34	0·002	0·62	0·03	
Adipose tissue	S25	25·3^{A,a}	22·6^{B}	18·6^{C}	19·1^{C}	14·5^{D}	<0·0001
	S50	23·9^{A,a,b}	21·9^{A,B}	21·4^{A,B}	17·5^{B}	14·2^{C}	<0·0001
	S100	21·5^{A,b,c}	21·9^{A}	21·3^{A}	20·4^{A}	14·9^{B}	<0·0001
	S200	20·6^{A,b,c}	22·5^{A}	21·2^{A,B}	20·9^{B}	14·9^{C}	<0·0001
	Effect of dose *P*	0·002	0·62	0·17	0·20	0·78	

^{a,b,c,A,B,C,D,E}Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).
*Σ2S represents the sum of SSS-, SSR-, SRS- and SRR-α-tocopherol.

tended to show the lowest bioavailability and RRS-α-tocopherol the highest bioavailability.

Distribution of stereoisomers of α-tocopherol in rats fed RRR-α-tocopheryl acetate

The effect of feeding different doses of RRR-α-tocopheryl-acetate to rats for 10 d on the distribution of α-tocopherol stereoisomers in plasma and tissues is shown in Table 6. On day 2, each of the four 2R isomers constituted 20–25 % of the total α-tocopherol content, whereas the four 2S forms constituted 5–16 %, with the lowest proportion in plasma and the highest in liver.

RRR- and RRS-α-tocopherol constituted in all cases the highest proportion of the 2R isomers. After 10 d of feeding with an increasing dietary dose of RRR-α-tocopheryl acetate, RRR-α-tocopherol was the dominating isomer. The highest increase in the proportion of RRR-α-tocopherol was observed in plasma, liver and spleen, whereas the substitution of the other isomers with RRR-α-tocopherol proceeded more slowly in lung and adipose tissue. With the exception of lung tissue in the N200 group, the substitution of the seven synthetic isomers with RRR-α-tocopherol was clearly dose dependent ($P<0·001$).

The absolute content of each of the four 2R isomers and the sum of the four 2S isomers in liver, lung, spleen and adipose tissue is presented in Table 7. Feeding RRR-α-tocopheryl acetate to the rats for 10 d generally caused an increase in the content of RRR-α-tocopherol and a decrease in the content of the other stereoisomers in the tissues analysed. In liver, RRR-α-tocopherol increased with increasing dietary dosage ($P<0·001$). In spleen, the absolute content of RRR-α-tocopherol was unaffected by the dietary treatment ($P=0·31$), and in lung tissue, the highest content of RRR-α-tocopherol was found in the N50 and N100 groups. Surprisingly, the lowest content of RRR-α-tocopherol was found in the N200 group. The synthetic 2R isomers of α-tocopherol decreased slightly with increasing dietary intake of RRR-α-tocopheryl acetate ($P<0·05$).

Adipose tissue behaved differently compared with plasma and the other tissues, because only the content of RRR-α-tocopherol changed upon feeding different amounts of RRR-α-tocopheryl acetate for 10 d, whereas the content of the other seven isomers remained constant and was independent of the dietary inclusion level of RRR-α-tocopheryl acetate ($P=0·14–0·59$). In all tissues, the absolute content of the four 2S isomers was unaffected by the dietary treatments ($P=0·49–0·96$).

Discussion

Measurements of the absorption of tocopherols from the diets are scarce, and great variations in the absorption coefficients have been reported, especially in studies in which lymphatic measurements have been used. Traber *et al.* (1986), in five rats with cannulated thoracic lymph ducts, measured an average absorption rate of α-tocopherol of 65 %, with a variation of 45–77 %, in the lymph. Porsgaard & Hoy (2000) showed

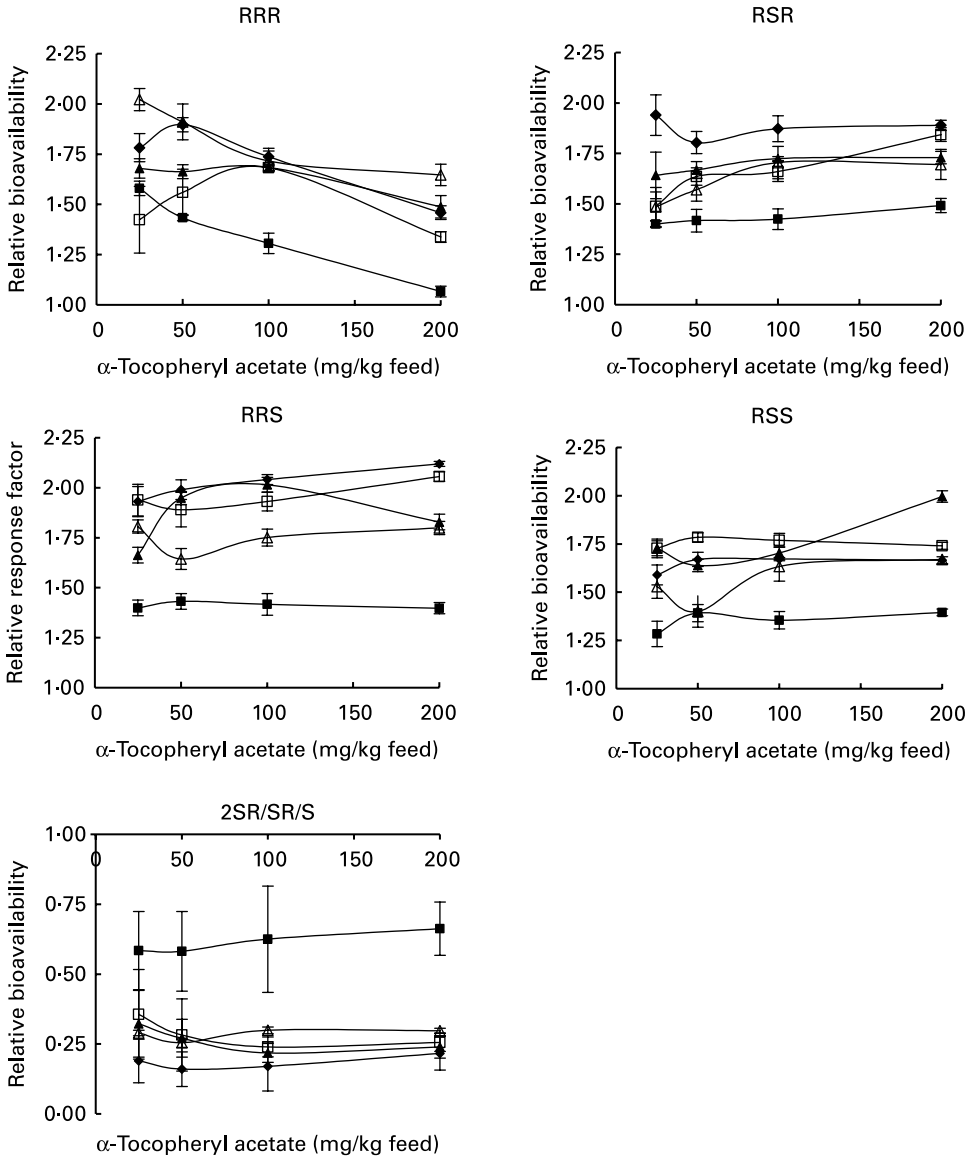

Fig. 2. Relative bioavailability compared with *all-rac*-α-tocopherol for stereoisomers of α-tocopherol in rats fed different concentrations of *all-rac*-α-tocopheryl acetate for 10 d. ◆, Plasma; ■, liver; ▲, lung; □, spleen; △, adipose tissue.

lymphatic absorption rates of α-tocopherol of 21, 45 and 79 % for α-tocopherol originating from sunflower, rapeseed or soyabean oil respectively. Liuzzi *et al.* (1998) reported apparent absorption coefficients of 80–85 % in intact rats by measuring the difference between intake and output in the faeces, these being in agreement with the absorption rates found in the present experiment. In experiments with broilers, apparent absorption coefficients of the same magnitude have been reported (Jensen *et al.* 1999; Knarreborg *et al.* 2004).

The average apparent absorption of RRR-α-tocopheryl acetate was significantly higher than the apparent absorption of *all-rac*-α-tocopheryl acetate. It was previously shown that the composition of bile salts modulates the stereoselectivity of carboxyl ester hydrolase, with a significant effect on the hydrolysis rate of the acetate esters of RRR-α-tocopherol and SRR-α-tocopherol (Zahalka *et al.* 1991; Moore *et al.* 1995). Likewise, a slightly higher faecal excretion of SRR-α-tocopherol compared with RRR-α-tocopherol has been shown in rats fed ^{14}C-labelled α-tocopherols (Kaneko *et al.* 2000). Thus, the present results suggest that RRR-α-tocopherol is slightly more bioavailable than *all-rac*-α-tocopheryl acetate at the intestinal level.

Four different doses of natural and synthetic α-tocopherol were compared in this study, and as seen from Table 4, the ratio between total α-tocopherol concentration measured in the plasma, lung and spleen of RRR-α-tocopheryl-acetate-fed rats compared with *all-rac*-α-tocopheryl-acetate-fed rats decreased with increasing dietary dose of the vitamin E, whereas no such effect could be obtained in liver and adipose tissue. In agreement with Blatt *et al.* (2004), this confirms that the relative bioavailability of *all-rac*- and RRR-α-tocopherols is not constant in rats, as well as in other animals and human subjects, because their relative concentrations vary between tissues and the amount of each dose, duration of dosing and time after

Table 6. Relative distribution of α-tocopherol stereoisomers in plasma and tissues from rats fed different levels of RRR-α-tocopheryl acetate for 10 d

		α-Tocopherol stereoisomers (%)*				
	Group	RRR	RRS	RSR	RSS	Σ2S*
Plasma	Initial†	26·1^{e}	25·8^{a}	21·3^{a}	21·4^{a}	5·4^{a}
	N25	69·3^{d}	11·5^{b}	9·8^{b}	9·4^{b}	0^{b}
	N50	80·0^{c}	7·7^{c}	6·2^{c}	6·1^{c}	0^{b}
	N100	88·8^{b}	4·5^{d}	3·3^{d}	3·4^{d}	0^{b}
	N200	92·8^{a}	0·7^{e}	2·0^{d}	4·5^{c,d}	0^{b}
	Effect of dose *P*	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001
Liver	Initial†	23·6^{d}	19·8^{a}	18·5^{a}	21·7^{a}	16·3^{a}
	N25	57·5^{c}	14·9^{b}	12·3^{b}	10·4^{b}	4·7^{b}
	N50	74·1^{b}	8·5^{c}	6·4^{c}	7·5^{c}	3·5^{b,c}
	N100	85·9^{a}	4·7^{d}	3·6^{d}	3·5^{d}	2·3^{b,c}
	N200	91·1^{a}	3·2^{d}	2·1^{d}	2·0^{d}	1·5^{c}
	Effect of dose *P*	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001
Lung	Initial†	27·7^{d}	20·8^{a}	22·7^{a}	18·6^{a}	10·3^{a}
	N25	56·3^{c}	13·0^{b}	8·0^{b}	13·8^{b}	8·8^{a}
	N50	71·4^{b}	8·5^{c}	5·4^{b,c}	8·4^{c}	6·3^{a,b}
	N100	81·4^{a}	5·5^{d}	3·7^{c,d}	6·1^{c}	3·3^{b}
	N200	77·4^{a,b}	3·9^{d}	2·0^{d}	7·2^{c}	9·5^{a}
	Effect of dose *P*	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001
Spleen	Initial†	26·9^{c}	23·8^{a}	20·5^{a}	20·2^{a}	8·6^{a}
	N25	75·0^{b}	9·5^{b}	7·1^{b}	8·0^{b}	0·4^{b}
	N50	76·8^{b}	9·2^{b}	6·7^{b}	6·4^{b}	0·9^{b}
	N100	88·0^{a}	4·8^{c}	2·3^{c}	4·0^{c}	0·9^{b}
	N200	90·7^{a}	4·0^{c}	0·9^{c}	3·4^{c}	0·9^{b}
	Effect of dose *P*	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001
Adipose tissue	Initial†	25·9^{d}	23·6^{a}	20·6^{a}	20·0^{a}	9·8^{a}
	N25	46·6^{c}	18·3^{b}	12·9^{b,c}	12·5^{b}	9·7^{a}
	N50	54·0^{b}	15·7^{c}	11·5^{b,c}	11·0^{b}	7·9^{a,b}
	N100	67·7^{a}	11·1^{d}	7·7^{c,d}	7·2^{c}	6·3^{b}
	N200	72·5^{a}	10·2^{d}	4·8^{d}	6·3^{c}	6·2^{b}
	Effect of dose *P*	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001

^{a,b,c,d,e} Mean values within a column marked with unlike superscript letters were significantly different ($P<0·05$).
* Σ2S represents the sum of SSS-, SSR-, SRS- and SRR- α-tocopherol.
† Initial values were from plasma and tissues from rats slaughtered on day 2 of the experiment.

dosing. However, in the first published rat resorption–gestation test (Harris & Ludwig, 1949), a dose-dependent difference in biological activity between natural and synthetic α-tocopherol was observed, and ratios between natural and synthetic vitamin E ranging from 1·03 to 1·81 were reported. In later reported rat resorption–gestation tests (Weiser & Vecchi, 1981, 1982; Leth & Søndergaard, 1983), a similar variation in relative biopotency was observed. However, apparently due to the variation in the results obtained, the average ratio of 1·36 (0·07) was established, and this biopotency factor, which has been officially accepted (United States Pharmacopeial Convention, 1979), does not take into account newer findings on vitamin E metabolism. According to Blatt *et al.* (2004) and confirmed by the present results, the relative bioavailability between *all-rac*- and RRR-α-tocopherols cannot be constant because their distribution and elimination involve processes that are saturable as well as stereospecific, and no dosage ratio can produce a constant ratio of effects in any process.

The chiral separation on HPLC of methyl ethers of the stereoisomers allows a more precise determination of the bioavailability and fate of each stereoisomer in the plasma and tissues. Thus, it is possible by this method to make a more precise differentiation in the stereospecific bioavailability and metabolism of natural and synthetic α-tocopherol than it is using biological responses such as resorption–gestation assays.

The chirality of the carbon atom at position 2 of the chromanol ring is the major determinant regarding the translocation of the different α-tocopherol stereoisomers (Behrens & Madére, 1991; Traber *et al.* 1993; Weiser *et al.* 1996). The highest proportion of 2S stereoisomers was observed in liver with up to one third of the total α-tocopherol, whereas the proportion of 2S stereoisomers in the other tissues analysed composed 12–15 % of the total. The liver is known to play a major role in the biodiscrimination between 2S- and 2R-α-tocopherols (Traber *et al.* 1990*a*,*b*; Kaneko *et al.* 2000) owing to the presence of the α-tocopherol transfer protein, which preferentially secretes the 2R forms into VLDL (Traber *et al.* 1990*a*,*b*), leaving the 2S forms back in the liver, from where they are metabolized and later secreted in the urine (Kaneko *et al.* 2000). α-Tocopherol transfer protein is present in the highest concentration in the liver and possesses stereoselectivity towards the R-configuration, mainly to the 2′ position in the chromanol ring but to a certain degree also to the 4′ and 8′ position of the side chain (Meier *et al.* 2003; Min *et al.* 2003). This mechanism is probably the reason why the 2S stereoisomer forms were present in high proportions in the liver relative to other tissues.

With the use of the present HPLC-technique, it was possible to study the change in the composition of the single 2R stereoisomer forms of the rats according to the dietary treatments. As can be seen from the present results, the proportion of

Table 7. Absolute content of α-tocopherol stereoisomers in liver, lung, spleen and adipose tissue from rats fed different levels of RRR-α-tocopheryl acetate for 10 d*

		α-tocopherol stereoisomers				
	Group	RRR	RRS	RSR	RSS	Σ2S*
Plasma (μmol/l)	N25	8·7^{c}	1·5^{a}	1·3^{a}	1·2^{a}	0
	N50	12·0^{b}	1·2^{b}	0·9^{b}	0·9^{b}	0
	N100	17·6^{a}	0·9^{b}	0·6^{b,c}	0·7^{c}	0
	N200	17·7^{a}	0·2^{c}	0·4^{c}	0·8^{b,c}	0
	Effect of dose	<0·0001	0·001	0·0002	0·005	
Liver (nmol/liver)	N25	13·6^{d}	3·4^{a}	2·8^{a}	2·4^{a}	1·1
	N50	24·8^{c}	2·8^{b}	2·1^{b}	2·5^{a}	1·1
	N100	41·7^{b}	2·2^{b,c}	1·8^{b,c}	1·6^{b}	1·1
	N200	61·3^{a}	2·1^{c}	1·51^{c}	1·4^{b}	1·0
	Effect of dose	<0·0001	0·001	0·0002	0·004	0·96
Lung (nmol/lung)	N25	17·6^{b}	4·0^{a}	2·5^{a}	4·4^{a}	2·7
	N50	28·8^{a}	3·4^{b}	2·1^{a}	3·3^{a,b}	2·5
	N100	32·4^{a}	2·1^{c}	1·5^{a}	2·6^{b,c}	1·4
	N200	13·6^{b}	0·7^{d}	0·3^{b}	1·2^{c}	1·6
	Effect of dose	0·001	<0·0001	0·003	0·005	0·49
Spleen (nmol/spleen)	N25	10·5	1·3^{a}	1·0^{a}	1·1^{a}	0·1
	N50	11·8	1·4^{a}	1·0^{a}	1·0^{a,b}	0·1
	N100	15·5	0·9^{a,b}	0·4^{b}	0·7^{b,c}	0·1
	N200	12·8	0·6^{b}	0·1^{b}	0·5^{c}	0·1
	Effect of dose	0·31	0·05	<0·0001	0·01	0·63
Adipose tissue (nmol/g)	N25	19·9^{b}	8·3	7·1	7·0	5·2
	N50	40·7^{a}	12·7	9·5	9·0	6·5
	N100	49·3^{a}	8·4	6·0	5·7	4·4
	N200	65·7^{a}	9·1	3·8	5·7	5·0
	Effect of dose	0·009	0·47	0·19	0·45	0·65

^{a,b,c,d,e} Mean values within a column marked with unlike superscript letters were significantly different ($P<0·05$).
*Σ2S represents the sum of SSS-, SSR-, SRS- and SRR- α-tocopherol.

the single 2R stereoisomers varied from one tissue to another, and the relative distribution reflected the difference in the turnover rate from one tissue to another. Thus, the non-constant relative bioavailability of the individual stereoisomers (Fig. 2) confirms that different dosages of *all-rac-* and RRR-α-tocopherol produce different ratios of the stereoisomers in all tissues. Accordingly, the present results are in contrast with those of Weiser *et al.* (1996), who reported equal bioavailability of the four 2R stereoisomers irrespective of an experimental feeding period with *all-rac*-α-tocopheryl acetate for 8 or 90 d. In addition, Blatt *et al.* (2004) concluded that the length of feeding and the dosage level also influenced the non-constant relative bioavailability.

In this context, it is an open question how the 'old' pool of α-tocopherol will affect the metabolism of newly absorbed tocopherol in the plasma and tissues. One disadvantage of the present method was that these pools could not be distinguished. In concordance with previous experiments (Ingold *et al.* 1987; Burton & Traber 1990; Traber *et al.* 1993; Blatt *et al.* 2004), the fastest shift towards RRR-α-tocopherol was seen in plasma, followed by liver, spleen and lung, with the adipose tissue as the slowest. However, the present results indicate that different stereoisomers remain in the different tissues for different periods. Thus, the 2S isomers remained in the tissues for a longer period than the 2R stereoisomers, and only a minor exchange of the isomers had taken place after 10 d on a RRR-α-tocopherol diet. At this point, adipose tissue contained the highest absolute content of 2S-α-tocopherol. It is noteworthy that, although the proportion of 2S-α-tocopherol decreased with increasing dietary dose of RRR-α-tocopherol (Table 6), the absolute content of 2S-α-tocopherol in liver, spleen, lung and adipose tissue was equal in groups N25–N200 (Table 7).

On the other hand, plasma, liver and adipose tissue showed the largest enrichment of RRR-α-tocopherol, whereas the RRR-α-tocopherol content of spleen and lung tissue was unaffected by the dietary dose of RRR-α-tocopheryl acetate. Thus, these results indicate that the RRR-α-tocopherol in adipose tissue is much more labile than previously reported (Ingold *et al.* 1987). In lung tissue, the lowest concentration of α-tocopherol was found in the N200 group, and the same tendency was seen in spleen. It is speculation whether this was a sign of overdosing with vitamin E. Analysis of the stereochemical composition of α-tocopherol (Tables 6 and 7) did not explain the lower concentration of RRR-α-tocopherol in lung tissue.

In addition to the studies on rats mentioned earlier, studies of human subjects (Acuff *et al.* 1994; Burton *et al.* 1998) and pigs (Lauridsen *et al.* 2002*a*,*b*) using ^{2}H-α-tocopherol have shown a 2:1 ratio of α-tocopherol concentrations when the subjects were fed an equal amount of *all-rac-* and RRR-α-tocopheryl acetate. Studies measuring the concentrations of the individual stereoisomers have generally found a 2:1 ratio of 2R:2S forms of α-tocopherol in the plasma (Weiser *et al.* 1996; Blatt *et al.* 2004), which also is in accordance with present study. However, Table 5 shows an inverse relationship between the bioavailability of RRR-α-tocopherol and the dietary dosage was observed (Fig. 2). On the other hand, the bioavailability of the synthetic stereoisomers was almost unaffected by different dietary doses. This result may indicate

a lack of regulatory mechanism of the synthetic isomers, or saturation of α-tocopherol transfer protein with RRR-α-tocopherol at high dietary levels of RRR-α-tocopheryl acetate (Arita *et al.* 1997; Hosomi *et al.* 1997).

In conclusion, the bioavailability of stereoisomers of α-tocopherol in rats depends on the dietary doses of *all-rac*- and RRR-α-tocopheryl acetate. Analysis of the stereochemical composition in fluids and tissues is a technically and economically valuable and relatively cheap tool for determining the bioavailability of vitamin E sources. The method seems to be applicable for targeting quantification of the difference between RRR- and *all-rac*-α-tocopherol, especially in nutritional studies with human subjects and animals, with which a large number of samples are required to overcome the individual difference.

Acknowledgements

Elsebeth Lyng Pedersen, Anna Stouby and Kathrine Høirup are greatly acknowledged for their technical assistance.

References

Acuff RV, Thedford SS, Hidiroglou NN, Papas AM & Odom TAJA (1994) Relative bioavailability of RRR- and *all-rac*-alpha-tocopheryl acetates in humans: studies using deuterated compounds. *Am J Clin Nutr* **60**, 397–402.

Arita M, Nomura K, Arai H & Inoue K (1997) Alpha-tocopherol transfer protein stimulates the secretion of alpha-tocopherol from a cultured liver cell line through a brefeldin A-insensitive pathway. *Proc Natl Acad Sci USA* **94**, 12437–12441.

Behrens WA & Madére R (1991) Tissue discrimination between dietary RRR-alpha- and *all-rac*-alpha-tocopherols in rats. *J Nutr* **121**, 454–459.

Blatt DH, Pryor WA, Mata JE & Rodriguez-Proteau R (2004) Re-evaluation of the relative potency of synthetic and natural α-tocopherol: experimental and clinical observations. *J Nutr Biochem* **15**, 380–395.

Burton GW & Traber MG (1990) Vitamin E: antioxidant activity, biokinetics, and bioavailability. In *Annual Review of Nutrition*, vol. 10, pp. 357–382 [RE Olson, DM Brier and DB McCormick, editors]. Palo Alto, CA: Annual Reviews.

Burton GW, Traber MG, Acuff RV, Walters DN, Kayden H, Hughes L & Ingold KU (1998) Human plasma and tissue alpha-tocopherol concentrations in response to supplementation with deuterated natural and synthetic vitamin E. *Am J Clin Nutr* **67**, 669–684.

Drotleff AM & Ternes W (2001) Determination of RS,E/Z-tocotrienols by HPLC. *J Chromatogr A* **909**, 215–223.

Harris PL & Ludwig MI (1949) Relative vitamin E potency of natural and of synthetic alpha-tocopherol. *J Biol Chem* **179**, 1111–1115.

Hosomi A, Arita M, Sato Y, Kiyose C, Ueda T, Igarashi O, Arai H & Inoue K (1997) Affinity for alpha-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. *FEBS Letts* **409**, 105–108.

Ingold KU, Burton GW, Foster DO, Hughes L, Lindsay DA & Webb A (1987) Biokinetics of and discrimination between dietary RRR- and SRR-alpha-tocopherols in the male rat. *Lipids* **22**, 163–172.

Jensen SK, Engberg RM & Hedemann MS (1999) *All-rac*-alpha-tocopherol acetate is a better vitamin E source than *all-rac*-alpha-tocopherol succinate for broilers. *J Nutr* **129**, 1355–1360.

Kaneko K, Kiyose C, Ueda T, Ichikawa H & Igarashi O (2001) Studies of the metabolism of α-tocopherol stereoisomers in rats using [5-methyl-14C]SRR- and RRR-α-tocopherol. *J Lipid Res* **41**, 357–367.

Kayden H & Traber MG (1993) Absorption, lipoprotein transport and regulation of plasma concentrations of vitamin E in humans. *Lipid Res* **34**, 343–358.

Knarreborg A, Lauridsen C, Engberg RM & Jensen SK (2004) Dietary antibiotic growth promoters enhance the bioavailability of α-tocopheryl acetate in broilers through mediations of the lipid absorption processes. *J Nutr* **134**, 1487–1492.

Lauridsen C, Engel H, Craig AM & Traber MG (2002*a*) Relative bioactivity of dietary RRR-alpha-tocopherol- and *all-rac*-alpha-tocopheryl acetates in swine assessed with deuterium-labeled vitamin E. *J Anim Sci* **80**, 1–6.

Lauridsen C, Engel H, Jensen SK, Craig AM & Traber MG (2002*b*) Lactating sows and suckling piglets preferentially incorporate RRR- over *all-rac*-alpha-tocopherol into milk, plasma and tissues. *J Nutr* **132**, 1258–1264.

Lauridsen C, Leonard SW, Griffin DA, Liebler DC, McClure TD & Traber MG (2001) Quantitative analysis by liquid chromatography-tandem mass spectrometry of deuterium-labeled and unlabeled vitamin E in biological samples. *Anal Biochem* **289**, 89–95.

Leth T & Søndergaard H (1983) Biological activity of *all-rac*-α-tocopherol and RRR-α-tocopherol determined by three different rat bioassays. *Int J Vit Nutr Res* **53**, 297–311.

Liuzzi JP, Cioccia AM & Hevia P (1998) In well-fed young rats, lactose-induced chronic diarrhea reduce the apparent absorption of vitamins A and E and affects preferentially vitamin E status. *J Nutr* **128**, 2467–2472.

Meier R, Tomizaki T, Schulze-Briese C, Bauman U & Stocker A (2003) The molecular basis of vitamin E retention: structure of human α-tocopherol transfer protein. *J Mol Biol* **331**, 725–734.

Min KC, Kovall RA & Hendrickson WA (2003) Crystal structure of human α-tocopherol transfer protein bound to its ligand: implications for ataxia with vitamin E deficiency. *PNAS* **100**, 14713–14718.

Moore ANJ, Dutton PJ, Zahalka HA, Burton GW & Ingold KU (1995) Bile salt-modulated stereoselection in the cholesterol esterase-catalyzed hydrolysis of alpha-tocopheryl acetates. *J Am Chem Soc* **117**, 5677–5686.

Porsgaard T & Hoy CE (2000) Absorption by rats of tocopherols present in edible vegetable oils. *Lipids* **35**, 1073–1078.

Scherf H, Machlin LJ, Frye TM, Krautmann BA & Williams SN (1996) Vitamin E biopotency: comparison of various 'natural-derived' and chemically synthesized alpha-tocopherols. *Anim Feed Sci Tech* **59**, 115–126.

Traber MG, Burton GW, Ingold KU & Kayden HJ (1990*a*) RRR- and SRR-alpha-tocopherols are secreted without discrimination in human chylomicrons, but RRR-alpha-tocopherol is preferentially secreted in very low density lipoproteins. *J Lipid Res* **31**, 675–685.

Traber MG, Cohn W & Muller DPR (1993) Absorption, transport and delivery to tissues. In *Vitamin E in Health and Disease*, pp. 35–51 [L Packer and J Fuchs, editors]. New York: Marcel Dekker.

Traber MG, Kayden HJ, Green JB & Green MH (1986) Absorption of water-miscible forms of vitamin E in a patient with cholestasis and in thoracic duct-cannulated rats. *Am J Clin Nutr* **44**, 914–923.

Traber MG, Rudel LL, Burton GW, Hughes L, Ingold KU & Kayden HJ (1990*b*) Nascent VLDL from liver perfusions of cynomolgus monkeys are preferentially enriched in RRR- compared with SRR-alpha-tocopherol: studies using deuterated tocopherols. *J Lipid Res* **31**, 687–694.

United States Pharmacopeial Convention (1979) *The National Formulary*. Rockville, MD: United States Pharmacopeial Convention.

Weiser H, Riss G & Kormann AW (1996) Biodiscrimination of the eight alpha-tocopherol stereoisomers result in preferential accumulation of the four 2R forms in tissues and plasma of rats. *J Nutr* **126**, 2539–2549.

Weiser H & Vecchi M (1981) Stereoisomers of α-tocopheryl acetate. II. Characterization of samples by physico-chemical methods and determination of biological activities in the rat resorption-gestation tests. *Int J Vitam Nutr Res* **51**, 100–113.

Weiser H & Vecchi M (1982) Stereoisomers of α-tocopheryl acetate. II. Biopotencies of all eight stereoisomers, individually or in mixtures, as determined by rat resorption-gestation tests. *Int J Vitam Nutr Res* **52**, 351–370.

Zahalka HA, Dutton PJ, O'Doherty B, Smart TAM, Phipps J, Foster DO, Burton GW & Ingold KU (1991) Bile salt modulated stereoselection in the cholesterol esterase catalyzed hydrolysis of alpha-tocopheryl acetates. *J Am Chem Soc* **113**, 2797–2799.