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Contents of conjugated linoleic acid isomers in ruminant-derived foods and estimation of their contribution to daily intake in Portugal

Susana V. Martins[1]*, Paula A. Lopes[1], Cristina M. Alfaia[1], Verónica S. Ribeiro[1,2], Teresa V. Guerreiro[1,2], Carlos M. G. A. Fontes[1], Matilde F. Castro[2], Graça Soveral[2,3] and José A. M. Prates[1]

[1]*Faculdade de Medicina Veterinária – CIISA, Av. da Universidade Técnica, Pólo Universitário do Alto da Ajuda, 1300-477 Lisbon, Portugal*
[2]*Faculdade de Farmácia – Centro de Estudos Farmacêuticos, Av. Professor Gama Pinto, 1649-003 Lisbon, Portugal*
[3]*Faculdade de Ciências e Tecnologia – REQUIMTE, 2829-516 Monte da Caparica, Portugal*

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The present study provides a detailed overview of the contents of conjugated linoleic acid (CLA) isomers in the most consumed Portuguese CLA-rich foods (milk, butter, yoghurt, cheese, beef and lamb meat), by using silver ion-HPLC. In addition, the contribution of these ruminant-derived foods to the daily intake of CLA isomers was estimated based on Portuguese consumption habits. The total CLA concentration in milk and dairy products ranged from 4·00 mg/g fat in yoghurt to 7·22 mg/g fat in butter, and, regarding meats, from 4·45 mg/g fat in intensively produced beef to 11·29 mg/g fat in lamb meat. The predominant CLA isomers identified in these products were *cis*-9,*trans*-11 (59·89–79·21 %) and *trans*-7,*cis*-9 (8·04–20·20 %). The average estimated total CLA intake for the Portuguese population was 73·70 mg/d. Milk and cheese are probably the two products with the highest contribution to the final CLA intake, as a result of their high fat content and consumption values. The results also suggested that *cis*-9,*trans*-11 and *trans*-7,*cis*-9 are the isomers most represented, with, respectively, 76·10 and 12·56 % of the total CLA intake. Being the first detailed report on the contents of total and individual CLA isomers in Portuguese commercial ruminant-derived foods, we further discuss the implication of the results for diet characteristics and human health.

Conjugated linoleic acid: Dairy products: Ruminant-derived meats: Silver ion-high-performance liquid chromatography

Conjugated linoleic acid (CLA) refers to a heterogeneous group of geometrical and positional isomers of linoleic acid (18: 2*n*-6) with conjugated double bonds. These double bonds can either be *trans* or *cis* configured, and a wide spectrum of isomers with variations in position (from 6,8- to 12,14-) and geometry (*trans*,*trans*, *trans*,*cis*, *cis*,*trans* and *cis*,*cis*) has been described[1,2]. Twenty different CLA isomers occur naturally in food, especially in ruminant-derived fat[3]. The major CLA isomer (*cis*-9,*trans*-11 (*c*9,*t*11), also known as rumenic acid), as well as the usually second most prevalent isomer (*trans*-7,*cis*-9; *t*7,*c*9), are produced in the rumen during microbial biohydrogenation of dietary 18: 2*n*-6 and in the tissues through Δ9-desaturation of the rumen-derived *trans*-octadecenoate (*trans*-11-18: 1)[4]. It is now accepted that the major contribution to these CLA isomers in ruminant-derived milk[5] and meat[6] is endogenous synthesis by Δ9-desaturation. With the exception of these two isomers, the origin of all other CLA isomers is supposed to arise from ruminal biohydrogenation of dietary unsaturated C18 fatty acids, even if the metabolic pathways are not yet elucidated[7].

Many experimental studies, using laboratory animals as well as human and cell-culture systems, suggest that CLA exhibits interesting biological activities: anticarcinogenic, anti-adipogenic, anti-diabetogenic, anti-atherogenic and anti-inflammatory[2]. The National Academy of Sciences of the USA recognised CLA as the only fatty acid that unequivocally inhibits carcinogenesis in experimental animals[8]. The mechanism of carcinogenesis modulation by CLA is not completely understood, although it may be related to its antioxidative properties or to the induction of apoptotic cell death and cell-cycle regulation[9]. Specific physiological effects have been linked to individual CLA isomers. The *trans*-10,*cis*-12 (*t*10,*c*12) isomer may play an important role in lipid metabolism, while the *c*9,*t*11 and the *t*10,*c*12 isomers seem to be equally effective in anticarcinogenesis[10]. Since individual CLA isomers have different biological activities, the determination of the CLA isomeric profile in ruminant-derived fat is required. However, recent supplementation studies in human subjects with the *t*10,*c*12-CLA isomer revealed some adverse effects. Risérus *et al.*[11] reported that diet supplementation with the *t*10,*c*12-CLA isomer increases oxidative stress and inflammatory biomarkers in obese men. In addition, the results obtained by Poirier *et al.*[12] showed that the *t*10,*c*12-CLA isomer can induce inflammation of white adipose tissue.

Abbreviations: CLA, conjugated linoleic acid; *c*9,*t*11, *cis*-9,*trans*-11; t7,c9, trans-7,cis-9; *t*10,*c*12, *trans*-10,*cis*-12; *t*11,*c*13, *trans*-11,*cis*-13.

* **Corresponding author:** Dr Susana V. Martins, fax +351 21 3652829, email smartins@fmv.utl.pt

Thus, as a natural dietary component, CLA isomers require special attention regarding the quantity consumed, and supplementation values remain a controversial issue.

Large differences in the values estimated for dietary total CLA intake among several populations have been reported (for a review, see Collomb *et al.*[13]). Although a range of strategies has been used to estimate total CLA intake, the rigorous assessment of CLA consumption requires documentation of its content and composition in the food supply[14]. It is well known that products from ruminant animals, including milk, dairy products and meat, are the most important sources of CLA in the human diet[15]. It is also well established that CLA isomers are also found in non-ruminant-derived meat, fish and plants, but at a much lower content[16]. In addition, crisps, chocolates, cakes and pastries have only negligible CLA values[15]. However, detailed information on CLA isomeric distribution in commonly consumed foods, which can only be achieved by silver ion-HPLC[17], is limited. Thus, from the above discussion it is clear that reliable information on CLA isomer consumption in human diets is highly required. Therefore, the goal of the present study was to assess the contents of total and individual CLA isomers in the most consumed ruminant-derived foods (milk, butter, yoghurt, cheese, beef and lamb meat) by the Portuguese population. In addition, based on the knowledge of Portuguese consumption habits, the contribution of these ruminant-derived foods to the daily intake of CLA isomers was also estimated.

Materials and methods

Reagents

Merck Biosciences (Darmstadt, Germany) supplied analytical-grade and liquid chromatographic-grade chemicals. Commercial standards of specific CLA isomers (*c*9,*t*11, *t*10,*c*12, *cis*-9,*cis*-11 and *trans*-9,*trans*-11) as methyl esters were obtained from Matreya Inc. (Pleasant Gap, PA, USA). Additional standards of CLA isomers (mixtures of *cis*,*trans*, *trans*,*cis* and *trans*,*trans* from positions 7,9 to 12,14) were synthesised as methyl esters, using the procedure described by Destaillats & Angers[18].

Sample collection and treatment

Different lots of the three most important commercial Portuguese brands[19] of half-fat milk (*n* 30), butter (*n* 30), yoghurt (*n* 45) and cheese (*n* 45) were obtained in a regular supermarket. Half-fat milk and Flamengo cheese were selected for the present study since they represent, respectively, 70 and 50 % of the correspondent product consumed by the Portuguese population[19]. Moreover, meats originated on different production systems were analysed. Beef samples were collected from young bulls produced in a typical intensive production system (*n* 14) and in a traditional (semi-extensive) production system according to Protected Designation of Origin (PDO) specifications (*n* 27). Finally, lamb-meat samples (*n* 8) were collected from animals reared in a typical extensive production system. All meat samples were removed from the ribeye portion (T1–T3) of animals' *longissimus dorsi*, 2–3 d after slaughter (+1°C), ground using a food processor (3 × 5 s), vacuum packed and stored at −70°C until required.

Meat and yoghurt samples were lyophilised (−60°C and 2·0 hPa) to constant weight using a lyophilisator (Edwards Modulyo; Edwards High Vacuum International, Crawley, West Sussex, UK), maintained exsiccated at room temperature and analysed within 2 weeks.

Lipid extraction and esterification

Lipid extraction from fresh (milk, butter and cheese) and lyophilised (meat and yoghurt) samples was performed using the procedures described by Fritsche *et al.*[20], except for milk whose extraction was based on the protocol described by Mir *et al.*[21]. Briefly, fat was extracted three times with methylene chloride–methanol (4:1, v/v) and a fourth time with *n*-hexane[20]. For milk, a volume of 6 ml was extracted with isopropanol (1 ×) followed by *n*-hexane (3 ×). Methyl esters of CLA isomers were obtained by base-catalysed transesterification[22] with sodium methoxide for 2 h at 30°C. Total lipids were measured gravimetrically, in duplicate, by weighing the fatty residue obtained after solvent evaporation.

Determination of individual conjugated linoleic acid isomers

The methyl esters of CLA isomers were individually separated by triple silver-ion columns in series (ChromSpher 5 Lipids, 250 mm × 4·6 mm internal diameter, 5 µm particle size; Chrompack, Bridgewater, NJ, USA), using an HPLC system (Agilent 1100 Series; Agilent Technologies Inc., Palo Alto, CA, USA) equipped with an autosampler and a diode array detector adjusted to 233 nm, according to the procedure reported previously[23]. The identification of the individual CLA isomers was achieved by comparison of their retention times with commercial and prepared standards, as well as with values published in the literature[24]. In addition, the identity of each isomer was controlled by the typical UV spectra of CLA isomers from the diode array detector in the range from 190 to 360 nm, using the spectral analysis of Agilent Chemstation for LC 3D Systems rev. A.09·01[25]. Total and individual CLA isomer contents in foods were determined based on the external standard technique (using *c*9,*t*11, *t*10,*c*12, *cis*-9,*cis*-11 and *trans*-9,*trans*-11 as representatives of each of the geometric groups of CLA isomers) and on the method of area normalisation[26]. The CLA isomers were expressed in gravimetric contents (mg/g product and mg/g fat) or as a percentage of the total CLA isomers identified (% of CLA isomers).

Estimation of the daily intake of conjugated linoleic acid isomers

The estimation of the daily intake of total and individual CLA isomers was calculated by multiplying the CLA contents (determined as described earlier) multiplied by the consumption values (per individual and d) of each product. The consumption values were obtained from national statistics, regarding the year 2003[27], and are presented in detail in Table 1.

Table 1. Estimation of the contribution of ruminant-derived foods to the average daily intake of total and individual conjugated linoleic acid (CLA) isomers in Portugal

	Milk and dairy products				Meats			
	Milk	Butter	Yoghurt	Cheese	Intensively produced beef	Traditionally produced beef	Lamb meat	Total
Daily consumption (g/day)	166·17*	4·38	54·25	27·12	47·15	1·07	8·77	
Daily CLA intake								
mg/d	18·01	11·57	3·23	29·61	3·04	0·09	8·16	73·70
%	24·38	15·66	4·37	40·10	4·12	0·12	11·07	100·00
CLA isomers/total CLA intake (%)								
trans-12,*trans*-14	0·22	0·13	0·04	0·45	0·02	<0·01	0·12	0·99
trans-11,*trans*-13	0·24	0·27	0·09	1·06	0·03	<0·01	0·20	1·89
trans-10,*trans*-12	0·13	0·24	0·07	0·44	0·05	<0·01	0·08	1·01
trans-9,*trans*-11	0·53	0·25	0·09	0·57	0·06	<0·01	0·24	1·73
trans-8,*trans*-10	0·30	0·15	0·04	0·26	0·01	<0·01	0·04	0·82
trans-7,*trans*-9	0·37	0·14	0·05	0·29	0·41	<0·01	0·06	1·32
trans-6,*trans*-8	0·11	0·06	0·02	0·12	0·00†	<0·01	<0·01	0·32
Total *trans,trans*	1·90	1·23	0·39	3·20	0·58	0·01	0·75	8·07
cis/trans-12,14	0·05	0·06	0·00†	0·00†	0·04	<0·01	0·07	0·23
trans-11,*cis*-13	0·29	0·13	0·06	0·89	0·06	<0·01	0·79	2·22
cis-11,*trans*-13	0·06	0·03	0·00†	0·04	0·03	<0·01	0·02	0·18
trans-10,*cis*-12	0·16	0·12	0·00†	0·01	0·13	<0·01	0·02	0·45
cis-9,*trans*-11	19·34	12·29	3·09	29·83	2·73	0·10	8·59	76·10
trans-7,*cis*-9‡	2·46	1·81	0·82	6·13	0·50	0·01	0·59	12·56
Total *cis/trans*	22·36	14·43	3·97	36·90	3·50	0·11	10·32	91·77
cis-11,*cis*-13	0·12	0·00†	0·00†	0·00†	0·00†	0·00†	0·00†	0·12
cis-10,*cis*-12	<0·01	0·00†	0·00†	0·00†	0·00†	0·00†	0·00†	0·00†
cis-9,*cis*-11	0·00†	0·00†	0·00†	0·00†	0·04	<0·01	0·00†	0·04
cis-8,*cis*-10	0·00†	0·00†	0·00†	0·00†	0·00†	0·00†	0·00†	0·00†
Total *cis,cis*	0·12	0·00†	0·00†	0·00†	0·04	<0·01	0·00†	0·16

* Values expressed in ml product/d.
† Missing calculation due to undetected isomer in CLA profile.
‡ This CLA isomer co-eluted with minor amounts of the *trans*-8,*cis*-10 isomer.

Results and discussion

Contents of conjugated linoleic acid isomers in commercial ruminant-derived foods

Data on the total (mg/g product) and specific (mg/g fat) CLA contents and its individual isomers (% of CLA isomers) in the most consumed Portuguese ruminant-derived foods are displayed in Table 2. The highest total CLA concentration was found in butter (2·64 mg/g product) and cheese (1·09 mg/g product) due to the high fat content present in these products. Lamb meat was the third richest product in total CLA (0·95 mg CLA/g meat). Yoghurt and intensively produced beef depicted residual CLA contents (0·06 mg/g product). CLA specific contents among meats ranged from 4·45 (intensively produced beef) to 11·29 mg/g fat (lamb meat). While milk showed a still remarkably high specific CLA content (7·22 mg CLA/g fat), the remaining products displayed considerably lower but similar concentrations, ranging from 4·00 to 4·97 mg/g fat. Lamb meat is usually originated from grass feeding systems and it is well known that the inclusion of grass in the diet improves CLA content in meat[28,29]. Compared with available data, Chin *et al.*[30] encountered a concentration of 5·5 mg CLA/g fat in homogenised milk. The specific CLA contents in butter and yoghurt were similar to those reported by Ma *et al.*[14] (4·7 and 4·4 mg/g fat, for butter and yoghurt, respectively). For yoghurt, Chin *et al.*[30] reported 4·8 mg CLA/g fat, whereas Lin *et al.*[31] found a concentration of 3·8 mg CLA/g fat. Regarding cheese, Shantha *et al.*[32] measured CLA contents varying from 3·2 to 8·9 mg/g fat, an interval that includes our calculated concentration of 4·86 mg CLA/g fat. Chin *et al.*[30] reported concentrations ranging from 2·7 mg CLA/g fat in veal to 4·3 mg CLA/g fat in fresh ground beef, this interval being close to the concentrations found in the present study for intensive and traditional meat samples. Also, Shantha *et al.*[33] described variations in CLA contents in raw steaks (ribeye, round, t-bone and sirloin) varying from 3·1 to 8·5 mg/g fat. The differences reported on CLA content in ruminant fatty acid composition may be explained by the influence of dietary factors (production system) and, to a lesser extent, by genetic factors[34]. French *et al.*[28] reported that meat fat from grazing steers displays higher CLA contents (10·8 mg/g fatty acids) than those obtained from animals fed concentrate (3·7 mg/g fatty acids). Additionally, discrepancies in the CLA content between different animal tissues, different breeds or upbringing, and even within the same breed, have already been reported and reviewed by Schmid *et al.*[35]. Regarding CLA in dairy products, Collomb *et al.*[7,36] concluded that the quality of milk and ripened cheese is influenced by many factors, including the composition of fodder consumed and the altitude at which the cow grazes.

The CLA isomeric profile of the analysed foods is presented in Table 2. In general, CLA distribution showed a clear predominance of the bioactive *c*9,*t*11 isomer (59·89–79·21 %), followed by the *t*7,*c*9 isomer (8·04–20·20 %), which co-eluted with minor amounts of the *trans*-8,*cis*-10 isomer. Similarly to total CLA content, diet is the major factor that affects the profile of CLA in ruminant fats[37,38]. Moreover, many of the differences in CLA profile appear to be related to pasture *v.*

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Table 2. Values of total (mg/g product) and specific (mg/g fat) conjugated linoleic acid (CLA) contents, and its isomeric distribution (% of CLA isomers), in the most consumed ruminant-derived milk, dairy products and meats by the Portuguese population

(Mean values and standard deviations)

	Milk and dairy products								Meats					
	Milk (*n* 30)		Butter (*n* 45)		Yoghurt (*n* 45)		Cheese (*n* 45)		Intensively produced beef (*n* 14)		Traditionally produced beef (*n* 27)		Lamb meat (*n* 8)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total content (mg/g product)	0·11*	0·045	2·64	0·789	0·06	0·022	1·09	0·313	0·06	0·050	0·08	0·052	0·95	0·196
Specific content (mg/g fat)	7·22	2·982	4·97	0·448	4·00	1·031	4·86	1·546	4·45	1·908	4·99	1·827	11·29	3·065
% of CLA isomers†														
trans-12,*trans*-14	0·92	0·193	0·99	0·492	0·82	0·228	1·02	0·457	0·55	0·422	0·48	0·346	1·10	0·200
trans-11,*trans*-13	1·00	0·395	1·99	0·981	1·87	0·341	2·43	0·890	0·57	0·352	1·03	0·258	1·80	0·512
trans-10,*trans*-12	0·52	0·455	1·45	0·310	1·69	0·452	1·20	0·635	1·04	0·480	0·59	0·651	0·74	0·121
trans-9,*trans*-11	2·21	3·159	1·58	0·267	2·04	0·244	1·49	0·330	1·16	0·598	2·14	0·926	2·17	0·082
trans-8,*trans*-10	1·29	1·225	0·91	0·244	1·02	0·237	0·74	0·364	0·37	0·501	0·38	0·268	0·37	0·045
trans-7,*trans*-9	1·46	2·579	0·87	0·152	1·10	0·098	0·79	0·248	15·03	14·075	0·81	0·725	0·56	0·091
trans-6,*trans*-8	0·46	0·352	0·35	0·100	0·47	0·109	0·35	0·210	n.d.	n.d.	0·23	0·411	0·04	0·050
Total *trans*,*trans*	7·85	3·932	8·13	1·116	9·01	0·709	8·02	0·532	18·71	13·122	5·65	1·434	6·77	0·605
cis/*trans*-12,14	0·18	0·105	0·31	0·326	n.d.	n.d.	n.d.	n.d.	1·21	1·232	1·35	1·615	0·64	0·157
trans-11,*cis*-13	1·21	0·309	0·68	0·546	1·06	0·893	2·13	0·801	1·26	1·765	1·22	1·228	6·86	2·543
cis-11,*trans*-13	0·23	0·120	0·16	0·251	n.d.	n.d.	0·10	0·169	1·10	1·761	0·72	0·974	0·15	0·129
trans-10,*cis*-12	0·64	0·205	0·64	0·624	n.d.	n.d.	0·01	0·099	3·79	2·017	2·12	1·703	0·22	0·333
cis-9,*trans*-11	79·21	8·442	77·77	2·857	69·74	4·822	73·70	5·288	59·89	13·683	78·35	6·315	77·31	2·027
trans-7,*cis*-9‡	10·21	8·917	12·32	3·188	20·20	5·359	16·03	5·846	12·09	5·206	9·17	3·885	8·04	4·364
Total *cis*/*trans*	91·67	3·950	91·87	1·116	90·99	0·709	91·98	0·532	80·21	13·818	92·93	1·974	93·23	0·605
cis-11,*cis*-13	0·48	0·206	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
cis-10,*cis*-12	< 0·01	< 0·01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
cis-9,*cis*-11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1·08	1·273	1·42	1·226	n.d.	n.d.
cis-8,*cis*-10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total *cis*,*cis*	0·48	0·207	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1·08	1·273	1·42	1·226	n.d.	n.d.

n.d., Not detected.
* Values expressed in mg/ml product.
† Values of each isomer as percentage of the total CLA isomers identified in each product.
‡ This CLA isomer co-eluted with minor amounts of the *trans*-8,*cis*-10 isomer.

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concentrate feeding. In the present study, lamb meat (pasture fed) showed higher percentages of the *trans*-11,*cis*-13 (*t*11,*c*13), *trans*-11,*trans*-13 and *trans*-12,*trans*-14 isomers and lower of the *t*7,*c*9 isomer, when compared with intensively and traditionally produced beefs. These differences may be explained by distinct grass intake of the animals since it was shown that pasture feeding, compared with concentrate feeding, increases the proportion of the *t*11,*c*13, *trans*-11,*trans*-13 and *trans*-12,*trans*-14 isomers and decreases the percentage of the *t*7,*c*9 isomer in beef lipids[37]. Based on these results, Dannenberger *et al.*[37] suggested that the *t*11,*c*13-, *trans*-12,*trans*-14- and *trans*-11,*trans*-13-CLA isomers are sensitive grass intake indicators. Breed and muscle type have also been reported as determinant of *t*11,*c*13 isomer percentage in beef lipids[37]. Regarding the CLA profile of intensively produced beef, the most abundant isomer was the *c*9,*t*11 (59·89 %) followed, in decreasing order, by the *trans*-7,*trans*-9 isomer (15·03 %) and the *t*7,*c*9 isomer (12·09 %). The *t*7,*c*9 isomer is mentioned frequently as the second most prevalent CLA isomer[39] and, like the most abundant *c*9,*t*11 isomer, its content in milk and tissues mainly results from endogenous synthesis through the Δ9-desaturation of the rumen-derived *trans*-octadecenoate precursor[6]. With the exception of the *c*9,*t*11 and *t*7,*c*9 isomers, the origin of all other CLA isomers is the ruminal biohydrogenation of dietary unsaturated C18 fatty acids, although the metabolic pathways producing these compounds are not yet elucidated[7]. The *t*10,*c*12 isomer, which apparently affects lipid metabolism[10], was present in very small proportions (0·01–2·12 %), reaching the highest levels in beef, from either intensive or semi-extensive production systems. In the *trans*,*trans* region, the most abundant isomer was the *trans*-9,*trans*-11, ranging from 1·16 to 2·21 % of total CLA. The sums of *trans*,*trans* (5·65–8·01 %) and *cis*/*trans* (90·99–93·23 %) CLA isomers were similar in all analysed products, except for the intensively produced beef, which were 18·71 and 80·21 % for *trans*,*trans* and *cis*/*trans*, respectively. The sum of the *cis*,*cis* CLA isomers only showed residual contents for all analysed products (< 1·42 %).

Estimation of the daily intake of conjugated linoleic acid isomers

Table 1 reports an estimation of the contribution of ruminant-derived foods for the daily intake of Portuguese consumers, per individual, of total and individual CLA isomers. Usually dietary intake is assessed by 3 or 7 d dietary records, representing the diet of a medium-term period, or by food-frequency questionnaires, expected to reflect the regular diet[40]. Additional to these methodologies, Ritzenthaler *et al.*[41] also conducted chemical analysis of food duplicates and concluded that daily CLA intake is underestimated by written dietary methods. In the present study, neither of these methodologies was applied. The calculation was done, as described earlier, by attending to the CLA contents determined in ruminant-derived products commercially available in Portugal (see Table 2) and the consumption data (per individual and d) of each product, according to the most recent national statistics[27]. The average daily intake of total CLA was estimated as being 73·70 mg/individual. This value may be slightly underestimated since non-ruminant-derived products (for example, meat, fish, plant crisps, chocolates, cakes and pastries) may have, as stated earlier, minor CLA contents[15,16]. The major food sources contributing to this value were cheese and milk (40·10 and 24·38 %, respectively), followed, in decreasing order, by butter (15·66 %) and lamb meat (11·07 %). Traditionally produced beef contributed only 0·12 % to the total CLA intake. Reflecting the food isomeric distribution, *c*9,*t*11 (76·10 %) and *t*7,*c*9 (12·56 %) were the main isomers present in the diet, as illustrated in Fig. 1. The third most important isomer was *t*11,*c*13, contributing 2·22 % to the daily intake. The *trans*-11,*trans*-13-, *trans*-9,*trans*-11- and *t*7,*t*9-CLA isomers were the most relevant in the *trans*,*trans* isomers region.

Depending on the country, the estimation of average CLA or *c*9,*t*11 consumption ranges between 15 and 1000 mg (Table 3). Even if the major food sources of CLA remain constant, their relative contributions to dietary intake may vary with food availability and eating preferences[35]. Based on milk consumption data, Wolff & Precht[42] estimated the *c*9,*t*11 ingestion in fifteen European countries, obtaining higher intake values in North Europe and lower in Mediterranean countries. According to these authors, France and Italy showed consumption values close to the European Union average and similar daily ingestions were observed for Spain (140 mg), Greece (150 mg) and Portugal (150 mg). The difference from our values for Portugal might be explained by the distinct estimation methods and statistics sources used. In Germany, the overall estimated *c*9,*t*11 consumption reported by Fritsche & Steinhart[16] was relatively high (350 and 430 mg/d for women and men, respectively), by 1-week dietary records. Based on the same method, an assessment of *c*9,*t*11 intake in a small group of young Canadians determined an average ingestion of 94·9 mg/d, ranging between 15 and 174 mg/d[43], which is not very far from our determination for this particular isomer (see Table 1). Ritzenthaler *et al.*[41] reported that young men and women living in the USA consumed about 151–212 and 140–193 mg/d for total CLA and *c*9,*t*11, respectively, by 3 d food duplicates. In agreement with that study, Herbel *et al.*[44] reported, for the same country and by using the same methodology, the daily intake of 127 mg CLA. McGuire *et al.*[45] advanced two possible reasons for the higher CLA intake for Germany compared with those for USA: more fat consumption in Germany and vast food nutrient database used. In Table 3, Australia shows the highest CLA consumption, 500–1000 mg/d[46], although the estimation method applied is unknown. Nutritional habits are also dependent on sex, although the lack of consumption statistics per woman and man did not allow taking this variable into account in the present study. Additionally, the present study differs from its counterparts presented in Table 3 since CLA concentration in food was determined by Ag^+-HPLC, in contrast to GC.

The importance of CLA in human nutrition is related to its anticarcinogenic activity[9]. Levels of CLA as low as 0·1 % in the diet have been seen as sufficient to produce a significant decrease in mammary tumour yield in rats challenged with a low dose of 7,12-dimethylbenz[a]anthracene[47]. The daily CLA intake in Portugal is 0·0038 % (intake of 1916·9 g food/d based on Portuguese statistics from Instituto Nacional de Estatística[48]), which only represents 3·8 % of the above recommended value. Moreover, at a 0·1 % dosage of CLA, a 300 g rat will consume approximately 0·015 g CLA/d. Extrapolating directly to a 70 kg man or woman, CLA consumption per d has to equal 3 g to confer a similar health

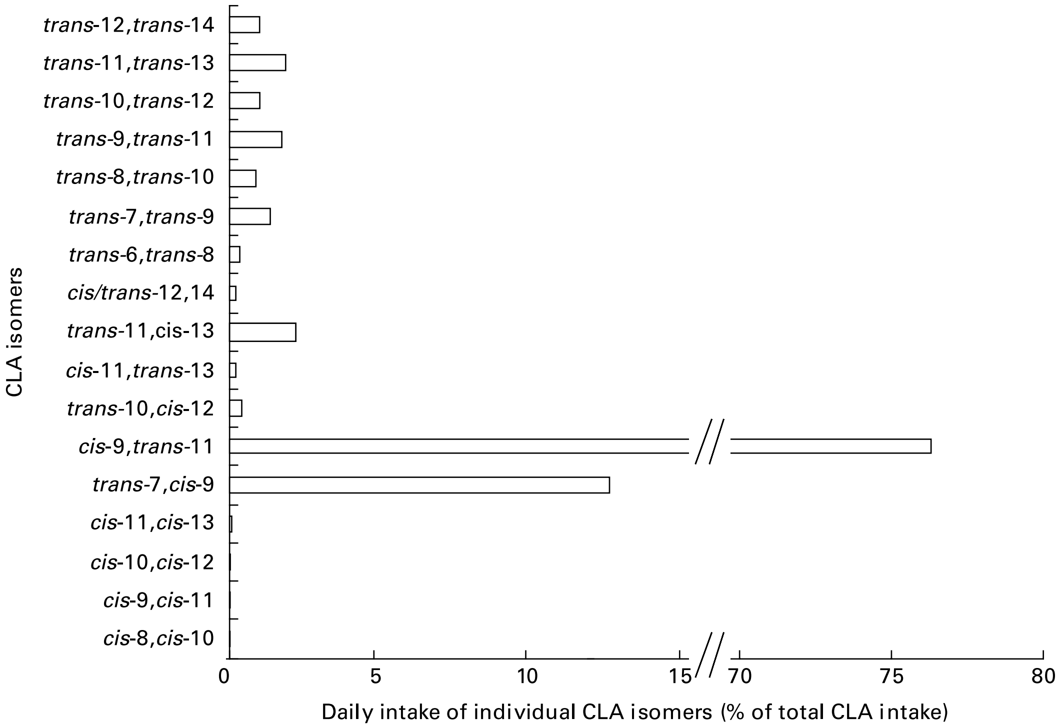

Fig. 1. Conjugated linoleic acid (CLA) isomeric distribution of the estimated daily CLA intake for the Portuguese population.

benefit[47]. However, differences in metabolic rate (particularly, in lipid metabolism) between these two species require a more suitable extrapolation from a rat model to man, using the metabolic weight[49]. Such extrapolation indicates that 0·8 g CLA/d would be protective in man. Therefore, on the basis of the anticancer effects of CLA in rats, as experimental models, a daily consumption of 0·8–3·0 g CLA might provide a significant human health benefit[50]. One of the few epidemiological studies relating the incidence of breast cancer in postmenopausal Netherlands women and levels of CLA intake showed a positive, although weak, correlation (P for trend =0·02) between 200 mg CLA intake per d and this cancer occurrence[51]. Another study associated high fat consumption, including CLA, with colorectal cancer incidence in Swedish women (P for trend =0·002) aged 40–76 years[52]. These authors concluded that high intakes of high-fat dairy foods, containing at least 127·8 mg CLA/d, may reduce the risk of colorectal cancer. Actually, in several countries, levels as high as the above-mentioned are consumed (see Table 3). However, it is well known that cancer is a multifactorial disease and, therefore, many other factors besides diet components still determine its occurrence. As being so, facing the relatively low ingestion value for Portugal presented in the present study (73·70 mg CLA/d), no preventive carcinogenic effects of CLA are expected to be found for the Portuguese population. A possible solution in order to reach beneficial values of CLA in the diet is through supplementation. Of note, dietary supplements have a different CLA isomeric profile compared with foodstuffs. The main difference concerns the high percentage of *t*10,*c*12 in supplements and some studies have recently demonstrated adverse effects of this isomer on human health[11].

Several authors have used indirect methodologies to estimate both typical and extreme intakes of CLA in a limited number of populations. However, in the present study we present an unparalleled and detailed overview of CLA isomeric profile in dairy and meat products, which allowed the direct estimation of daily CLA intake for the Portuguese population. In human trials, synthetic CLA supplements are usually used and these do not reflect the natural isomeric composition in foodstuffs. Whether natural CLA sources (meat, milk and its derivatives from ruminant animals) have a similar impact on human health warrants further research (for a review, see Schmid *et al.*[35]). Essentially, examination of the relationships among dietary intake of CLA isomers, their contents in adipose tissue and plasma and risk of various chronic degenerative diseases (for example, cancer, diabetes, and atherosclerosis) is essential for scientists and public health officials to draw conclusions concerning the importance of dietary CLA (and its isomers) to human health. Studies are actually in progress to clarify these questions. Therefore, enhancing our knowledge concerning CLA isomeric profile in various populations must remain a primary focus for research in this area.

Conclusions

In the present study, contents of total and individual CLA isomers in various Portuguese ruminant-derived foods were assessed. Regarding milk and dairy products, total CLA contents ranged from 4·00 mg/g fat in yoghurt to 7·22 mg/g fat in butter, while in meats these concentrations varied from 4·45 mg/g fat in intensively produced beef to 11·29 mg/g fat in lamb meat. The most abundant CLA isomers in these

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Table 3. Average conjugated linoleic acid (CLA) intake (mg/d) estimated for several countries

Country	Method*	Daily intake	Estimated isomer	Reference
European Union	Milk intake	250	*cis*-9,*trans*-11	Wolff & Precht (2002)[42]
Portugal	Milk intake	150	*cis*-9,*trans*-11	Wolff & Precht (2002)[42]
Spain	Milk intake	140	*cis*-9,*trans*-11	Wolff & Precht (2002)[42]
France	Milk intake	300	*cis*-9,*trans*-11	Wolff & Precht (2002)[42]
Italy	Milk intake	220	*cis*-9,*trans*-11	Wolff & Precht (2002)[42]
Greece	Milk intake	150	*cis*-9,*trans*-11	Wolff & Precht (2002)[42]
Germany	7 d DR	350–430	*cis*-9,*trans*-11	Fritsche & Steinhart (1998)[16]
Sweden	1 d DR	160	*cis*-9,*trans*-11	Jiang *et al.* (1999)[53]
Canada	7 d DR	15–174	*cis*-9,*trans*-11	Ens *et al.* (2001)[43]
USA	FFQ	93–197	Total CLA	Ritzenthaler *et al.* (2001)[41]
USA	FFQ	72–151	*cis*-9,*trans*-11	Ritzenthaler *et al.* (2001)[41]
USA	3 d FD	151–212	Total CLA	Ritzenthaler *et al.* (2001)[41]
USA	3 d FD	140–193	*cis*-9,*trans*-11	Ritzenthaler *et al.* (2001)[41]
USA	3 d DR	104–176	Total CLA	Ritzenthaler *et al.* (2001)[41]
USA	3 d DR	79–133	*cis*-9,*trans*-11	Ritzenthaler *et al.* (2001)[41]
USA	3 d DR	127	*cis*-9,*trans*-11	Herbel *et al.* (1998)[44]
Australia	Not reported	500–1000	Total CLA	Parodi (1994)[46]

DR, dietary records; FD, food duplicates; FFQ, food-frequency questionnaires.
* The methods for CLA intake estimation were based on either the estimation of milk intake (milk intake), DR, FD or FFQ.

products were *c*9,*t*11 (59·89–79·21 %) and *t*7,*c*9 (8·04–20·20 %). The average total CLA intake for the Portuguese population was calculated to be 73·70 mg/d. Moreover, milk and cheese are believed to be the two products that contributed the most to the final CLA intake. Finally, as a result from the food isomeric distribution, the *c*9,*t*11 and *t*7,*c*9 are the most represented CLA isomers, with, respectively, 76·10 and 12·56 % of the total intake value.

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