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A comparison of CLA intake and source between female and male Italian students

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Abstract.
BACKGROUND: CLA isomers were proposed as factors affecting human health, although it is nowadays not clear if positively or not. The consciousness of CLA amount in foods is thus needed.
OBJECTIVE: To investigate the CLA intake between male and female and the role of each food as CLA source and to complete the database on CLA content in Italian foods.
METHODS: A three-days food questionnaire was administered to a cohort of 40 healthy students ranging from 19 to 25 years old. Data from the food diaries were combined with the database on CLA content in foods.
RESULTS: In our cohort the calculated average daily CLA intake was 130.8 mg overall: males daily consumed 164.86 mg of CLA, while females 96.65 mg. Cheese accounted for more than 62% of CLA intake ($P < 0.05$). Milk is the second food for contribution to CLA intake (16.25 mg/day; $P < 0.05$), while yoghurt, meat and confectionery showed a minor contribution (8.17, 5.15, and 4.91 mg/day, respectively; $P < 0.05$).
CONCLUSIONS: CLA intake in our cohort was lower than value proposed as biologically relevant (3 g/day). Dairy foods were the most relevant sources of CLA in both sexes.

Keywords: Food diary, CLA intake, CLA source, dairy products, meat products

1. Introduction

Conjugated linoleic acid (CLA) isomers *cis9,trans11* and *trans10,cis12* are fatty acids naturally present in ruminant food products as intermediates in the biohydrogenation of linoleic acid by rumen bacteria. *c9,t11* isomer is also produced by vaccenic acid desaturation in the mammary gland [1, 2] covering from 75 to 90% of total CLA in milk [3]. CLA content in milk and meat depends mainly on the feed regimen: in fact pasture was reported as a CLA enhancing factor, due to its high polyunsaturated fatty acid (PUFA) content, that are CLA precursors [4, 5]. Also species, breed, age and individual conditions could influence CLA content. As a matter of fact ewe milk is the richest in CLA among all milk types [6]. Moreover CLA content in food can be affected by the food manufacturing: for example cooking temperatures, ripening period and microbial starter selection could influence the final CLA amount [7, 8].

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According to animal and cell culture experiments, *c9,t11* plays a protective role against cancer and atherosclerosis [9] and can also attenuate insulin resistance [10, 11].

Other experiments have demonstrated that diet supplementation with CLA can increase body weight loss in obese subjects fed on a hypocaloric diet [12, 13].

However Benjamin et al. [14] recently reviewed the biological effects of high dosages CLA supplementation and reported some detrimental effects (increase of oxidative stress, irritation of intestine, insulin resistance) observed in clinical trials where CLA was given as supplement at dosage ranging from 1.7 to 8.0 g/d. Furthermore CLA supplementation did not always resulted in a reduction of body weight and body fat, so the evidence of an improvement in weight loss rate due to CLA supplementation cannot be considered fully convincing [14, 15].

Anyway CLA is a molecule that can affect human metabolism, then the estimation of CLA intake and CLA source is needed.

Several studies were performed to estimate the CLA daily intake in human subjects from different countries, and a strong variation in CLA intake among different countries was found. In Germany Fritsche and Steinhart [16] using data from a national dietary survey estimated a daily CLA intake in man and women respectively of 440 and 360 mg/d. In the same year Ritzenthaler et al. [17] published US data from 3-days dietary records and calculated a CLA intake of 104 mg/d CLA for women and 176 mg/d for men. The variation in daily intake among different countries may be due to dietary behavior and preferences, food processing, animal feeding. However also the method used to estimate the food intake could explain these discrepancies.

Based on recent anticancer researches, Ip et al. [18] proposed 3 g/day as the lowest daily CLA intake to obtain anti-carcinogenic effects. Most of the studies on CLA intake however reported intakes very far from this value.

To our knowledge, no comprehensive information about CLA intake in Italy is nowadays available. Thus, in order to properly investigate the role of CLA in disease and in prevention, a complete database of CLA-foods is required. We carried out previous studies to determine the content of CLA in dairy products from both bovine and ovine milk, and in meat products [19–22].

In the present study, the CLA content of cakes, puddings and spoon-desserts were determined with the aim to complete the CLA database. Moreover, we estimated for the first time in Italy the daily *c9,t11* intake in a cohort of healthy Italian students, also comparing CLA intake between male and female, and investigating which foods are the most relevant source of CLA in both sexes.

2. Methods

2.1. Subjects and study design

The study was conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures involving human subjects were approved by the Università Cattolica Ethical Committee (protocol number 24475/14) and registered on ClinicalTrials.gov as “Dietary Conjugated Linoleic Acid (CLA) Intake Among Students in Italy” (n°NCT02335762).

Written informed consent was obtained from all participants. 40 healthy subjects were recruited using advertisements posted at the Catholic University of Piacenza, between January and October 2012. To be eligible for inclusion, participants had to be between the ages of 19 and 25 years-old, self-reported healthy and not suffering disorders of lipid metabolism and from eating disorders. We excluded subjects who were pregnant or breast-feeding, subjects consuming an energy-restricted diet and/or vegan or vegetarians. Height (using a stadiometer) and weight (using a Gima scale) of each subject were measured and the body mass index (BMI) was calculated (kg/m^2). Subjects were asked to eat as usual in subsequent 3 days to emulate as best their habits and they were educated to report also place, time, number and quantity of serving portions consumed using a validated diary previously applied in the Italian National Survey INRAN-SCAI [23]. If they could not weight exactly each portion, a photographic atlas developed for the Italian component of the European Prospective Investigation into Cancer and Nutrition (EPIC) study [24] and Moli-Sani Project [25] were used. At the end of the three days the food diaries were checked and for each subject CLA and energy intakes were estimated.

Table 1
Average CLA isomers content (mg/g fat) in confectionaries

Confectionary	*c9,t11* CLA	
	(mg/g fat)	*(mg/100 g prod.)*
Milk chocolate bar with cream and wafer	0.02	0.74
Milk chocolate bar 1	1.18	41.3
Milk chocolate bar 2	1.18	41.3
Milk chocolate tab with cereals	0.99	32.67
Sponge cake covered and filled with milk chocolate	1.18	34.22
Wafer	n.d.	n.d.
Chocolate brioche	n.d.	n.d.
Plum Cake	0.17	3.57
Chocolate sponge cake	0.06	1.02
Cream brioches	3.00	51.00
Vanilla pudding	5.12	14.07
Chocolate pudding	4.66	21.39
Cream caramel	5.17	27
Ice cream	n.d.	n.d.

n.d, not detected.

2.2. *Database of CLA content of foods*

The complete list of food consumed by subjects was identified and it was linked to the CLA contents per 100 g of product. We used CLA data for dairy products and meat products published by Prandini et al. [19, 20] and Cicognini et al. [21, 22] as reported in Table 1.

Since confectionary products (main commercial snacks available in Italian market) were not included in the previous paper, they were purchased in September 2012 in North-Italian large-scale retail trade and analysed for CLA content.

Fat content, lipid extraction, CLA isomers methyl esters preparation and GC analysis were conducted as previously reported in Cicognini et al. [21, 22].

The estimation of the mean daily intake of CLA was calculated by multiplying the CLA content for each food by the consumption values of each food items.

2.3. *Statistical analysis*

The analysis was performed with the Statistical Analysis Systems version 9.3 (SAS Institute, Cary, NC, USA). For total CLA intake, the simple effect of sex was tested and means were *post-hoc* compared by the t Student test. Successively, an ANOVA was carried out and fixed effects in the model were sex ($n = 2$), food ($n = 7$), and their first order interaction. Being first order interaction significant, the differences between foods were analyzed separately for each sex. The multiple comparisons between means was performed using the Bonferroni test.

3. Results

Data on the content of CLA (mg/g product) for cakes, puddings and spoon-desserts are reported in Table 2. Cream caramel and vanilla and chocolate pudding contained more *c9,t11* than the other confectionaries, 5.17, 5.12 and 4.66 mg/g fat respectively; brioches filled with cream contained 3.00 mg/g fat. The *t10,c12* was not detected.

Table 2
Average daily *c9,t11* intake in male ($n = 16$) and female ($n = 24$)

Food	CLA (mg/day)		CLA (%)
	M	*F*	
Cheese	115.70[a]	65.79[a]	61.93[a]
Milk	20.57[b]	11.93[b]	13.19[b]
Yoghurt	9.97[b]	6.37[b]	7.51[bc]
Confectionery	7.33[b]	2.96[b]	3.16[bc]
Meat	7.51[b]	2.31[b]	3.45[bc]
Olive oil	1.66[b]	6.45[b]	3.61[bc]
Ham	2.12[b]	0.98[b]	1.15[c]
√MSE	91.44		13.99
Significance of:			
Food	<0.0001		<0.0001
Sex	0.0094		ns
Sex*Food	0.0029		ns

[a,b]Means in the same column with different superscripts differ ($P < 0.05$). When Food × Sex interaction was significant, the differences were evaluated within each Sex. CLA, conjugated linoleic acid intake (mg/day) from each food. CLA %, % of conjugated linoleic acid introduced with each food calculated on total CLA intake. √MSE, root mean square error.

Table 3
Average CLA intake (mg/d) estimated in studies from several countries

Year	Country	Daily intake	Estimated isomer	Method	Authors
2010	UK	97.5	*c9,t11*	3d dietary record	Mushtak (2010)
2001	Canada	94.9	*c9,t11*	7d dietary record	Ens (2001)
2007	Portugal	73.7	*c9,t11*	National dietary survey	Martins (2007)
1998	Germany	400	*c9,t11*	7d dietary record	Fritshe & Steinhart (1998)
1994	Australia	500–1500	Total CLA	*Unknown method*	Parodi (1994)
2001	USA	79–133	*c9,t11*	3d dietary record	Rizenthaler (2001)
1999	Sweden	350–430	*c9,t11*	1d dietary record	Jiang (1999)

3.1. Estimation of the mean daily intake and main source of CLA

The entire study population consisted in 40 participants (16 male and 24 female). They were all Caucasian, mean age of 22 years, BMI of female was 22.5 (SD 3.6), with a body fat percentage of 30.0% (SD 5.6%) and was 22.5 (SD 1.7) in male and body fat percentage was 16.0% (SD 3.4%). The estimated average energy intake of subjects was 10803 and 7932 kJ/day (2582 and 1896 kcal/day), respectively for males and females.

In our cohort the calculated average daily CLA intake was 130.8 mg overall and a significant difference in the CLA intake of males and females was found (Table 2). Males consumed 164.86 mg of *c9,t11*, while females 96.65 mg/d daily ($P < 0.05$). Cheese accounted for more than 60% of CLA intake and can be recognised as the main source of CLA for young people. Milk is the second food for contribution to CLA intake (16.25 mg/day), while yoghurt, meat and confectionery showed a minor contribution (8.17, 4.91 and 5.15 mg/day, respectively).

Moreover in Table 2 also data on the contribution of different food groups on CLA intake are reported.

4. Discussion

Regarding the CLA amount in confectionaries, it is interesting to note that data on the content of CLA (mg/g fat) for cream caramel and vanilla or chocolate puddings showed more *c9,t11* than the others (5.17, 5.12 and 4.66 mg/g fat respectively). Brioches filled with cream followed with 3.00 mg CLA/g fat mainly due to the milk used as ingredient.

Even if brioches or milk chocolate tabs contained less CLA as mg/g fat than puddings and cream caramel, they however showed higher values as mg/100 g of product, due to the high level of fat.

Regarding the evaluation of CLA consumption, the average daily CLA intake was 130.8 mg overall and 164.86 in male and 96.65 in female (Table 2). The main source of CLA in this population is cheese. Cheeses accounted for about 62% of CLA intake and can be recognised as the main source of CLA for Italian young people.

As expected, men consume more energy than women, similarly to what is reported in the most recent national food consumption survey [26]. The mean energy intake of our population is in line on what published by Sette et al. [26] (10000 kJ for men, and 8113 kJ for women). The gender differences found can be due to the total amount of food intake, as well as to the food behaviour. Consequently males introduced significantly higher amounts of *c9,t11* from cheeses (115.70 mg/g fat) and cold cuts (2.12 mg/g fat) than females (respectively 65.79 and 0.98 mg/g fat) (Table 2). CLA intakes from other foods were instead similar in the two sexes.

Depending on the country, the estimation of average CLA consumption vary widely as reported in Table 3 and these data suggest that the intake in our population is far from the recommended daily intake proposed by Ip et al. [18]. However, due to the endogenous conversion of 20% of dietary vaccenic acid to CLA in human subjects [27], this gap should likely be lower. As a matter of fact dairy products contain about twofold vaccenic acid than CLA, thus the conversion factor to the real CLA intake can be hypothesized as 1.4.

In our cohort the calculated average daily CLA intake was 164.86 mg/d in males and 96.65 mg/d in females, similar to the estimate published by Ritzenthaler et al. [17], who reported a consumption of 133 mg/d for male and 104 mg/day for female in the U.S. population using a 3-d dietary record. A lower daily dietary intake was instead reported by Mushtaq et al. [28] and Ens et al. [29]. The first study showed CLA intakes of 126.7 and 68.3 mg/d in males and females, respectively in the UK (average 97.5 mg/d); in the second study, they found a mean daily CLA intake of 94.9 mg using dietary records in a small group of young Canadians. Martins et al. [30] using a national consumption survey estimated a very low daily intake of 73.7 mg/d of CLA in Portugal. This estimate is almost half of ours and as reported by authors may be due to the estimation method, the Portuguese eating preferences and the HPLC method used to determine the CLA concentration in contrast to GC.

In Germany, a higher daily CLA intake ranging from 440 to 360 mg/day in men and women, respectively, was reported by Fritshe and Steinhart [16] using a national dietary survey. However, the highest level of CLA intake worldwide was reported by Parodi et al. [31].

The Australian study showed a range of the intake between 500 and 1500 mg/day although the estimation method applied is unknown. These levels probably depend on individual's dietary preferences and seasonal factors affecting CLA in milk and ruminant fat.

The major differences in CLA intake between countries are related to the factors affecting CLA contents in food, e.g. animal feeding (grazing increase CLA levels in milk and meat), animal species (milk and meat from sheep are richest in CLA than similar products obtained from bovine or goats).

In our cohort cheeses are the main sources of CLA for 62% of total intake. These data are consistent with Ritzenthaler et al. [17] and Jiang et al. [32]. In the first work dairy foods were the main source of CLA, while in the second a significant positive relationship between milk fat intake and CLA content of adipose tissue was reported. Also in the work of Voorips et al. [33] dairy foods were the main source of CLA, even if butter accounted for almost the 30% of CLA intake, while butter is scarcely consumed in our population.

Our study had several strengths. It is, to our knowledge, the first study to evaluate the CLA intake in free-living Italian subjects. The role of CLA on human health has gained attention. Moreover, strength relates to the method used for the estimation of CLA. A greater amount of detail can be recorded using the food record because it is open ended, and its compilation do not rely on the respondents' memory, therefore some errors may be minimized. Portion size was estimated using household utensils and food pictures. Other indirect methodologies can be used to measure the CLA intake, but they can affect the estimates, as reported in some National Surveys using Food Frequency Questionnaire [30]. Another important aspect is the database of CLA-foods developed by our group. As a matter of fact, the application of the same published analytical method for all the samples included in the database ensured the homogeneity of data.

Our study had however some limitations. The number of participants is relatively small, limiting the statistical power of the study. Moreover, we instructed participant to record all food and beverages consumed, though such instrument is not completely reliable. Although it is widely used as intake indicator among groups of people, it can underestimate the mean intake of CLA as reported by Ritzenthaler et al. [17].

5. Conclusions

Current findings showed that CLA intake in a cohort of University Italian students was lower than value proposed by Ip et al. [18] as biologically relevant, the average calculated CLA daily intake being on average 130.8 mg and 164.9 mg in male and 96.7 mg in female. In addition, present data suggested that dairy foods are the most relevant sources of CLA for both sexes. Therefore, even if further studies are required to understand if the level of CLA considered biologically relevant should effectively be reached, dairy foods will play a role in the total dietary CLA intake.

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