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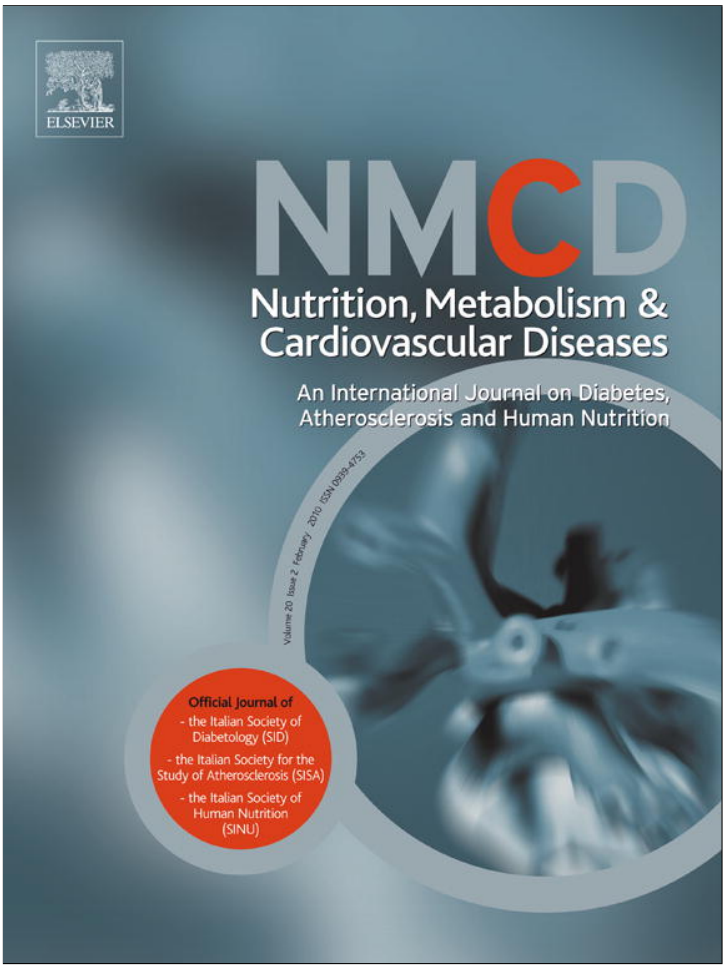

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Effects of a dairy product (pecorino cheese) naturally rich in *cis*-9, *trans*-11 conjugated linoleic acid on lipid, inflammatory and haemorheological variables: A dietary intervention study

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Abstract *Background and aim:* Some studies recently reported a favourable effect for *cis*-9, *trans*-11 conjugated linoleic acid (CLA) on plasma lipoprotein profile of healthy subjects. Aim of this crossover intervention study was to evaluate the influence of a short-term dietary intake of a cheese derived from sheep's milk naturally rich in CLA on several atherosclerotic biomarkers, in comparison with a commercially available cheese.
Methods and results: Ten subjects (6 F; 4 M) with a median age of 51.5 followed for 10 weeks a diet containing 200 g/week of cheese naturally rich in CLA (*intervention period*) and for the same period a diet containing a commercially available cheese of the same quantity (*placebo period*). Consumption of the dairy product naturally rich in *cis*-9, *trans*-11 CLA determined a significant ($p < 0.05$) reduction in inflammatory parameters such as interleukin-6 (pre: 8.08 ± 1.57 vs. post: 4.58 ± 0.94 pg/mL), interleukin-8 (pre: 45.02 ± 5.82 vs. post: 28.59 ± 2.64 pg/mL), and tumour necrosis factor-α (pre: 53.58 ± 25.67 vs. post: 32.09 ± 17.42 pg/mL) whereas no significant differences in the placebo period were observed. With regard to haemorheological parameters, the test period significantly ameliorated erythrocytes' filtration rate (pre: 7.61 ± 0.71% vs. post: 9.12 ± 0.97%; $p = 0.03$) with respect to the placebo period. Moreover, a reduction in the extent of platelet aggregation, induced by

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arachidonic acid [pre: 87.8 ± 1.76% vs. post: 77.7 ± 3.56%; $p = 0.04$] was observed during the test period in comparison with the placebo period.
Conclusions: Dietary short-term intake of the tested dairy product naturally rich in *cis*-9, *trans*-11 CLA appeared to cause favourable biochemical changes of atherosclerotic markers.

Introduction

Conjugated linoleic acid (CLA) is a mixture of linoleic acid isomers with conjugated double bonds [1]. This polyunsaturated fatty acid is produced naturally in the rumen of ruminant animals and can be found in foods derived from ruminant sources. Indeed, the major dietary source of CLA for humans is meat, fat, milk and dairy products of ruminant animals [1–3]. Recent studies showed beneficial effects of an isomer of CLA, the *cis*-9, *trans*-11, that accounts for as much as 85–90% of the total CLA content in food products, on neoplastic and atherosclerotic processes, as well as on immune function and modulation of body composition [4–6]. To date, the contribution of CLA to the human diet is relatively small; thus, over the years growing interest in the potential for naturally increasing the isomer *cis*-9, *trans*-11 CLA content of milk and dairy products has been reported [7]. Up to now, however, relatively little information from dietary intervention studies with CLA-enriched dairy products in humans is available.

Aim of this crossover intervention study was to investigate the effect of consumption of a sheep cheese naturally rich in *cis*-9, *trans*-11 CLA compared to the effect of a commercially available cheese on lipid parameters, inflammatory cytokines, haemorheological variables and platelet function of clinically healthy subjects.

Methods

Study population

Ten clinically healthy subjects (6 F; 4 M; mean age: 45.6 years) were recruited. Subjects taking any prescribed medications, food supplements, or following special diets were excluded from the study. Participants were subjects who reported being fond of cheese and willing to undertake a cheese consumption period. In order to identify symptom-free subjects and to exclude who were suspected of having any form of vascular and inflammatory diseases, a detailed interview addressed to personal and familial history was performed. Current smoking status was determined at the time of physical examination. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. All participants gave signed informed consent; the study was approved by the local Ethics Committee and applies with the Declaration of Helsinki.

Cheese characteristics

Pecorino Toscano and placebo cheeses were commercial products and they were bought together in a store. These two cheeses were ripened respectively at 90 and 160 days, as suggested by disciplinary of production.

Ether extract and crude protein content of the two cheeses were determined according to the Association of Official Analytical Chemists [8]. Pecorino cheese showed a significantly higher content of crude protein and ether extract with respect to placebo cheese (pecorino: 39.0 and 32.5 g/100 g of dry vs. placebo cheese 27 g and 28 g; $p < 0.05$ for all).

Cheese fat, for fatty acid profile, was extracted according to Folch et al. [9]. The fatty acid methyl esters (FAME) were prepared with a base catalyzed trans-esterification according to Christie [10]. The FAME were separated on GC equipped with a capillary column (CP-Select CB for FAME Varian, Middelburg, The Netherlands: 100 m × 0.25 mm i.d.; film thickness 0.20 μm) and quantified using nonadecanoic acid (C19:0), methyl ester (Sigma Chemical Co., St. Louis, MO) as the internal standard. The injector and flame ionization detector temperatures were respectively 270 °C and 300 °C. The programmed temperature was 40 °C for 4 min, increased to 120 °C at a rate of 10 °C/min, maintained at 120 °C for 1 min, increased to 180 °C at a rate of 5 °C/min, maintained at 180 °C for 18 min, increased to 200 °C at a rate of 2 °C/min, maintained at 200 °C for 1 min, increased to 230 °C at a rate of 2 °C/min and maintained at this last temperature for 19 min. The split ratio was 1:100 and helium was the carrier gas with a flux of 1 mL/min. Individual FAME were identified by comparison of the relative retention times of FAME peaks from samples, with those of the standard mixture 37 Component FAME Mix (Supelco, Bellefonte, PA). Individual *trans*-9 C18:1, *trans*-11 C18:1, *trans*-12 C18:1, *trans*-13 C18:1 (Supelco), individual *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA (Matreya Inc.), CLA mix standard (Sigma Chemical Co) and published isomeric profile [11,12] were used to identify *trans*-C18:1 and CLA isomers of interest. Nonadecanoic acid was used as an internal standard to avoid overestimation biases that can arise when results are expressed as a relative percentage of the area of analysed peaks (since areas of small peaks are not included). Since all methods using peak normalization and expressing results in relative percentage of the area of analysed peaks are subjected to an over-evaluation, because areas of small peaks are not considered, the problem was avoided using nonadecanoic acid as the internal standard. All results concerning the fatty acid composition are expressed as g/100 g of lipids. The fatty acid profile of the two cheeses is shown in Table 1.

Experimental design

The study had a crossover design. Before starting, a run-in period (i.e. a period when the clinical trial has begun but the dietary intervention has not started) for all the subjects was performed. After that period, all participants were

instructed to undertake a 10-week study period (*intervention period*) with about 2 kg of the test cheese (200 g/week). An expert dietician gave specific dietary recommendations to consume cheese three times per week by maintaining a regular eating pattern during the intervention period, except for cheese intake. The amount of cheese to be consumed was provided at the end of the physical examination to each participant, in weighed portion size at no cost. Thereafter, a washout period (i.e. a period when subjects were instructed not to consume any type of cheeses) of 10 weeks was followed by all the subjects (*washout period*). For the next 10 weeks of the study (*control period*) a commercially available cow cheese of the same quantity of the test cheese was given to all the subjects. Fasting blood samples were obtained at the beginning and at the end of each period.

Blood measurements

Venous blood samples anticoagulated with 0.129 M sodium citrate (ratio 9:1) were collected from the antecubital vein into evacuated plastic tubes (Vacutainer), after an overnight fasting. Whole venous blood was also collected in tubes without anticoagulant. Citrated and serum samples were centrifuged at 2000 g for 10 min at 4 °C, and supernatants were stored in aliquots at −80 °C until assays. Lipid variables were assessed by conventional methods. Interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-12 (IL-12), tumour necrosis factor-α (TNF-α) and vascular endothelial growth factor (VEGF) levels were determined by using the Bio-Plex cytokine assay (Bio-Rad Laboratories Inc., Hercules, CA, USA), according to manufacturer's instructions.

With regard to haemorheological variables, whole blood viscosity (WBV) and plasma viscosity (PLV) were measured using the rotational viscosimeter LS 30 (Contraves, Zurich, Switzerland), whereas erythrocytes' filtration was measured by a microcomputer-assisted filtrometer, model MF4 (Myrenne GmbH, Roetgen, Germany). Erythrocyte deformability index (DI) was estimated by a curve indicating erythrocyte filtration throughout a 10 min recording in order to determine rheological properties of erythrocytes, passing them through polycarbonate filters with 5-μm micropores (Nucleopore, Pleasanton, CA, USA). The initial flow rate from the microcomputer-generated curves was used for assessing erythrocyte DI. WBV was analysed at shear rates of 0.512 s^{-1} and 94.5 s^{-1}.

For platelet aggregation assays whole blood specimens were centrifuged for 10 min at 250 g to obtain platelet-rich plasma. Afterwards, platelet-rich plasma was stimulated with 2 and 10 μM ADP (Mascia Brunelli, Milan, Italy) and with 1 mM arachidonic acid (Sigma-Aldrich, Milan, Italy) using an APACT 4 aggregometer (Helena Laboratories Italia s.p.a., Milan, Italy). The coefficient of variation of arachidonic acid and ADP platelet aggregations were 5.8% and 6.8%, respectively.

Table 1 Profile of the main fatty acids in to the two cheeses (g/100 lipids).

Fatty acid	Pecorino cheese	Placebo cheese	*p* value
C8	2.02	1.14	<0.05
C10	6.32	2.80	<0.05
C12	3.71	3.87	n.s.
C14	8.58	11.82	<0.05
C14:1	0.12	0.28	<0.05
C15	0.94	1.00	n.s.
C16	17.53	32.56	<0.05
C16:1n7	0.86	1.53	<0.05
C17	0.46	0.47	n.s.
C18	8.48	9.57	<0.05
C18:1 *trans*-6 + *trans*-7 + *trans*-8	0.19	0.21	n.s.
C18:1 *trans*-9	0.25	0.31	<0.05
C18:1 *trans*-10	0.43	0.19	<0.05
C18:1 *trans*-11	3.28	0.40	<0.05
C18:1 *cis*-9	14.65	15.44	<0.05
C18:1 *cis*-11	0.26	0.28	n.s.
C18:1 *cis*-12	0.09	0.16	n.s.
C18:2n-6 *trans*	0.12	0.09	n.s.
C18:2n-6 *cis*	0.92	1.84	<0.05
C18:3n-6	0.05	0.11	<0.05
C18:3n3	0.62	0.43	<0.05
C20	0.15	0.19	n.s.
cla cis-9, *trans*-11	1.56	0.19	<0.05
C18:4n3	0.04	0.05	n.s.
C21	0.06	0.07	n.s.
C20:4n6	0.06	0.05	n.s.
C20:5n3	0.06	0.03	n.s.
C23	0.03	0.02	n.s.
C24	0.03	0.01	n.s.
C24:1	0.01	0.01	n.s.
C22:6n3	0.03	0.02	n.s.

n.s. not significant.

Statistical analysis

Statistical analysis was performed by using the SPSS (Statistical Package for Social Sciences Inc., Chicago, IL, USA) software for Windows (Version 13.0). Results are expressed either as mean ± standard deviation (SD) or as median and range, as appropriate. All data referable to the single different treatments were analysed by means of the one-way ANOVA, keeping the factor ''diet'' as the fixed one. The analyses were simplified by calculating the absolute change for each variable tested (mean value at baseline subtracted from the mean value after intervention for each subject) with independent *t* sample tests. No carry-over effect was observed. Therefore, all data were treated as paired samples from a crossover study. Data that were not normally distributed were logarithmically transformed or analysed by the nonparametric Wilcoxon signed-ranked test. Data were analysed by using paired *t* tests for significant differences between changes observed during test and control intervention periods. Moreover, in order to compare the effect of test cheese compared to baseline and to the placebo cheese a general linear model for repeated measurements, after adjustment for age and gender, was performed. A p-value < 0.05 was considered to indicate statistical significance.

Table 2 Demographic and baseline characteristics.

	Males ($n = 4$)	Females ($n = 6$)	p
Age, years[a]	53 (30–65)	56 (30–63)	0.09
BMI, kg/m^2	24.8 ± 4.2	25.2 ± 3.8	0.8
Smoking habit, n (%)	1 (25)	2 (33.3)	0.8
Total cholesterol, mg/dL	176.2 ± 17.4	195.7 ± 32.7	0.4
LDL-cholesterol, mg/dL	97.5 ± 16.2	111 ± 26.8	0.6
Triglycerides, mg/dL	75 ± 11.5	89.7 ± 22.2	0.5

BMI, Body Mass Index; LDL, Low-Density Lipoprotein.
[a] Median and (range).

Results

Baseline and demographic characteristics of the subjects enrolled in the study are shown in Table 2. The median age of the study population was 51.5 years (range: 30–65). Three subjects were current smokers. There were no significant differences for age, body mass index, and lipid variables between males and females.

Changes of parameters investigated after the 2 different dietary interventions were measured by using a general linear model for repeated measurements. At a model adjusted for age and gender lipid variables (total, LDL, and HDL cholesterols, triglycerides) showed no significant changes during either intervention phases (Table 3).

On the contrary, the intervention period determined significant decreases for inflammatory cytokines (Table 4). Indeed, the study participants reported significant changes of interleukin-6 (−43.2%), interleukin-8 (−36.5%), and tumour necrosis factor-alpha (−40.1%) levels during the test period compared to the control period.

Haemorheological variables were also investigated before and after the two different dietary interventions (Table 5). After adjustment for age, gender, DI resulted to be significantly improved at the end of the intervention period, whereas no significant changes during the control period were observed. In addition, changes in PLV during the intervention period resulted to be significantly different from those observed during the control period.

Finally, platelet aggregation induced by different agonists at different concentrations was also investigated in relation to the two dietary interventions (Table 6). At the end of the intervention period a significant reduction of platelet aggregation induced by arachidonic acid was observed, whereas no changes during the control period were reported.

Discussion

The present study examined the effects of a natural *cis*-9, *trans*-11 CLA-rich ewe cheese on several human health parameters. Main findings were the significant reductions of inflammatory cytokines, rheological parameters and platelet aggregation profile during the dietary intervention with CLA-rich cheese compared to a dietary intervention with a commercially available cow cheese. Indeed, dietary intervention with pecorino cheese obtained by milk of ewes fed with a grass naturally rich in *cis*-9, *trans*-11 CLA determined a consistent reduction of inflammatory cytokines, a relevant improvement of rheological conditions of blood, and a substantial reduction of platelet aggregation

Table 3 Effect of dietary interventions with CLA-rich and control dairy products on lipid profile

Variable	Test period	Control period	p[a]
Total cholesterol, mg/dL			
Before	187.9 ± 8.3	195.7 ± 9.79	
After	189.2 ± 11.9	195.7 ± 7.08	
p (before–after)	0.9	0.1	
Change	1.3 (−17.42; 20.02)	0 (−25.8; 25.8)	0.8
LDL-cholesterol, mg/dL			
Before	105.6 ± 7.39	123.6 ± 9.02	
After	116.2 ± 8.89	127.5 ± 7.01	
p (before–after)	0.06	0.6	
Change	10.6 (−0.35; 21.55)	3.87 (−15.55; 23.31)	0.7
HDL-cholesterol, mg/dL			
Before	64.2 ± 4.24	56.3 ± 3.14	
After	53.7 ± 3.04	52.1 ± 2.54	
p (before–after)	0.09	0.2	
Change	−10.5 (−23.21; 2.26)	−4.12 (−10.87; 2.62)	0.4
Triglycerides, mg/dL			
Before	75.8 ± 14.87	79.88 ± 11.1	
After	85.8 ± 21.29	80.75 ± 7.06	
p (before–after)	0.08	0.7	
Change	10 (6.52; 26.52)	0.87 (−25.19; 26.95)	0.7

LDL: Low-Density Lipoprotein; HDL: High-Density Lipoprotein.
General linear model adjusted for age, and gender; values are expressed as geometric mean and SD.
[a] Comparison between absolute changes induced by the 2 diets (Wilcoxon signed-ranks test).

Table 4 Effect of dietary interventions with CLA-rich and control dairy products on inflammatory profile

Variable	Test period	Control period	p^a
Interleukin-6, pg/mL			
Before	8.08 ± 1.57	6.07 ± 1.55	
After	4.58 ± 0.94	6.64 ± 1.56	
p (before—after)	0.015	0.8	
Change	−3.49 (−6.08; −0.91)	0.57 (−5.97; 7.11)	0.036
Interleukin-8, pg/mL			
Before	45.02 ± 5.82	31.37 ± 5.58	
After	28.59 ± 2.64	27.42 ± 4.62	
p (before—after)	0.012	0.3	
Change	−16.42 (−28; −4.84)	−3.95 (−12.13; 4.24)	0.021
TNF-α, pg/mL			
Before	53.58 ± 25.67	21.22 ± 11.86	
After	32.09 ± 17.42	18.64 ± 9.58	
p (before—after)	0.049	0.4	
Change	−21.48 (−42.89; −0.06)	−2.59 (−10.95; 5.78)	0.018
VEGF, pg/mL			
Before	959.24 ± 114.36	901.99 ± 242.30	
After	842.42 ± 566.79	601.41 ± 157.25	
p (before—after)	0.5	0.09	
Change	−116.81 (−485.81; 252.18)	−300.59 (−672.49; 71.31)	0.07
Interleukin-10, pg/mL			
Before	19.75 ± 6.63	10.84 ± 3.78	
After	11.41 ± 2.79	7.16 ± 1.88	
p (before—after)	0.3	0.3	
Change	−8.34 (−27.27; 10.59)	−3.68 (−11.40; 4.05)	0.8
Interleukin-12, pg/mL			
Before	49.5 ± 27.93	15.75 ± 4.19	
After	25.76 ± 9.66	22.13 ± 16.81	
p (before—after)	0.3	0.06	
Change	−23.75 (−32.88; 80.37)	6.38 (−27.73; 40.49)	0.9

TNF, Tumour Necrosis Factor; VEGF, Vascular Endothelial Growth Factor.
General linear model adjusted for age, and gender; values are expressed as geometric mean ± SD.
[a] Comparison between absolute changes induced by the 2 diets (Wilcoxon signed-ranks test).

induced by arachidonic acid. All these modifications were not observed in the control period of dietary intervention with a commercially available cow cheese, so resulting in a significant difference between the test ewe cheese and the cow cheese in terms of reduction of the global risk of cardiovascular diseases. Actually, sheep cheese is naturally rich in CLA because it is made with milk from grazing ewes. In fact, it is well known from the literature that it is possible to manipulate the fatty acid profile of sheep dairy products, in a higher extent than that of the cows, to maximize the content of beneficial fatty acids by the use of appropriate fresh forage-based regimens [13].

CLA has been recently reported to have antiatherogenic and antineoplastic properties [4—6]. In particular, one of the CLA isomer, the *cis*-9, *trans*-11, has been reported to have beneficial effects on atherogenesis, although only few studies on humans are available [14—16]. One of the few available studies is a recent paper by Tricon et al. that examined the effect of naturally enriched dairy products with *cis*-9, *trans*-11 CLA on blood profile of healthy middle-aged men [16].

The present dietary intervention study was conducted in order to evaluate the effects of diet containing a *cis*-9, *trans*-11 CLA-rich cheese on different parameters, including lipid parameters, so reflecting the complex pathophysiology of atherothrombotic diseases. However, similar to Tricon et al. [16], who reported no effects of such dairy product on lipid parameters, we were not able to report any significant effects of the test cheese on lipid parameters. To date, the effects on lipid metabolism of *cis*-9, *trans*-11 CLA are still objects of concern. LDL-cholesterol appears to be significantly reduced by this particular isomer of CLA [15]. Conversely, triglycerides did not significantly change or decrease compared to placebo in some studies with CLA supplementation [16]. Similarly, the effect of CLA supplementation on HDL-cholesterol is unclear. Some studies have shown a decrease in HDL-cholesterol with mixed isomer CLA supplementation, one study found an increase in HDL-cholesterol [14], and others reported no effect [15,17]. In another study that examined the effect of supplementation with the individual isomer *cis*-9, *trans*-11, no effect on HDL-cholesterol was observed after 8 week of supplementation [4]. Thus, it appears that the effects of this particular isoform of CLA on lipid profile need to be further examined to assess the atherogenic potential of CLA supplementation in humans.

Table 5 Effect of dietary interventions with CLA-rich and control dairy products on haemorheological profile

Variable	Test period	Control period	p^a
Red blood cells, 10^{12}/L			
Before	4.58 ± 0.12	4.77 ± 0.16	
After	4.66 ± 0.16	4.74 ± 0.15	
p (before–after)	0.4	0.6	
Change	0.08 (−0.12; 0.28)	−0.04 (−0.16; 0.11)	0.21
Hematocrit, %			
Before	40.8 ± 0.95	41.4 ± 0.98	
After	40.9 ± 1.03	41.2 ± 0.90	
p (before–after)	0.9	0.5	
Change	0.1 (−1.9; 2.2)	−0.2 (−1.5; 0.9)	0.42
WBV 94.500 s^{-1}, mPa s			
Before	4.24 ± 0.04	4.26 ± 0.05	
After	4.26 ± 0.03	4.20 ± 0.10	
p (before–after)	0.8	0.4	
Change	0.02 (−0.006; −0.82)	−0.06 (−0.34; 0.17)	0.34
WBV 0.512 s^{-1}, mPa s			
Before	23.77 ± 0.35	22.86 ± 0.80	
After	24.33 ± 0.78	22.16 ± 0.93	
p (before–after)	0.5	0.3	
Change	0.56 (−1.13; 2.25)	−0.70 (−2.45; 1.05)	0.41
EF, %			
Before	7.61 ± 0.71	9.64 ± 0.44	
After	9.12 ± 0.97	9.41 ± 0.55	
p (before–after)	0.027	0.7	
Change	1.51 (0.23; 2.29)	−0.22 (−2.14; 1.69)	0.027
PLV, mPa s			
Before	1.33 ± 0.01	1.32 ± 0.04	
After	1.31 ± 0.02	1.36 ± 0.03	
p (before–after)	0.1	0.09	
Change	−0.02 (−0.04; 0.06)	0.04 (−0.01; 0.09)	0.03

WBV, Whole blood viscosity; EF, Erythrocyte filtration; PLV, Plasma viscosity.
General linear model adjusted for age, and gender; values are expressed as mean ± SD.
[a] Comparison between absolute changes induced by the 2 diets (Wilcoxon signed-ranks test).

A striking evidence of our study is, on the other hand, the significant effect of *cis*-9, *trans*-11 CLA cheese on inflammatory, haemorheological and platelet aggregation profiles, by possibly hypothesising a beneficial role of this particular isoform of CLA on these pathophysiological processes. CLAs have been previously reported to attenuate inflammatory cytokine expression in animals and humans [18], and it has been recently reported that they are able to inhibit the expression of cytokine-induced adhesion molecules on endothelial and smooth muscle cells [19]. Thus, it is conceivable to hypothesise that CLAs are able to attenuate the atherosclerotic process through inhibition of the initiating inflammatory cytokines, such as those measured in our study, as well as through inhibition of the stress signalling cascades these cytokines elicit. On the other hand, it must be noted that a recent study conducted in postmenopausal women reported no significant effects of an oil mixture with *cis*-9, *trans*-11 CLA on inflammatory markers, as compared with olive oil [17].

Another important finding of the study is the significant improvement of red cell deformability obtained in the group of subjects consuming the test cheese in comparison with those consuming the commercial cheese. Actually, red cell deformability was reported to be significantly influenced by omega-3 polyunsaturated fatty acids; we have indeed recently observed a beneficial effect of fish eating on these specific risk markers [20]. On the other hand, CLAs from ruminant animals and omega-3 polyunsaturated fatty acids from fish oil share some striking effects on cell functions, on animal models of specific diseases as well as on human health [4]. These include beneficial effects in inhibiting cancer cell proliferation, anti-tumour effects in animal models of cancer, anti-inflammatory effects on cells, in animal models and humans, and more recently for CLAs, beneficial effects in cardiovascular disease prevention. Hence, possible similar effects of CLAs on red blood cells can be hypothesised. On the other hand, Wahle et al. recently suggested a hypothesis of the link between these CLA and omega-3 polyunsaturated fatty acids [1]. Therefore, the findings observed in our crossover intervention study, where a consumption of a cheese naturally rich in CLAs demonstrated to have a beneficial effect on circulating levels of some atherosclerotic risk markers, like omega-3 polyunsaturated fatty acids have, seem to

Table 6 Adjusted values for platelet function's parameters

Variable	Test period	Control period	p^a
Aggregation ADP 2 μmol, %			
Before	79.7 ± 2.60	78.3 ± 5.69	
After	81.7 ± 2.32	83.5 ± 4.50	
p (before–after)	0.7	0.2	
Change	2 (−8.99; 12.99)	5.25 (−5; 15.50)	0.7
Aggregation ADP 10 μmol, %			
Before	85.4 ± 1.66	90.38 ± 1.06	
After	84.6 ± 3.67	91.75 ± 3.03	
p (before–after)	0.9	0.7	
Change	−0.8 (−10.64; 9.04)	1.38 (−6.15; 8.89)	0.6
Aggregation collagen, %			
Before	81.2 ± 1.71	82.63 ± 10.09	
After	82 ± 3.41	89.88 ± 2.93	
p (before–after)	0.8	0.5	
Change	−0.8 (−10.18; 8.58)	7.25 (−17.48; 31.98)	0.6
Aggregation arachidonic, %			
Before	87.8 ± 1.76	83.1 ± 3.22	
After	77.7 ± 3.56	88.8 ± 0.78	
p (before–after)	0.045	0.09	
Change	−10.1 (−19.91; −0.29)	5.62 (−1.17; 12.42)	0.02

ADP, Adenosine diphosphate.
General linear model adjusted for age, and gender; values are expressed as mean ± SD.
[a] Comparison between absolute changes induced by the 2 diets (Wilcoxon signed-ranks test).

hypothesise a mechanism for CLA similar to that reported for omega-3 compounds.

Interestingly, in our limited study population we were able to report significant reduced levels of aggregation induced by arachidonic acid after the consumption of test cheese. The role of platelets and platelet aggregation on the occurrence of atherothrombotic diseases is well known for decades, and the clinical significance of a high level of aggregation on the risk profile of the general population is of arousing interest [21]. In the present study, we observed a significant reduction of platelet aggregation induced by arachidonic acid in our population of clinically healthy subjects, after the intervention period with the test cheese. The beneficial effects observed by this particular type of fatty acid can be possibly linked to the common pathways between CLAs and arachidonic acid for the cyclooxygenase reaction and for the synthesis of prostaglandins. Therefore, it is likely to suggest a possible beneficial role of this particular type of fatty acids on the synthesis of arachidonic acid, and, consequently, on the biochemical processes related to arachidonic acid such as platelet aggregation.

Our study has several main limitations. An important limitation is the restricted sample size of the study. Further and larger studies need to be conducted before drawing any firm conclusion on the effects of such food products on human health. The results of the present study are just a promising basis for evaluating more completely this aspect of clinical nutrition.

Another limitation is the lack of assessment of dietary habits and physical activity in our study population. The possibility that changes in dietary and/or lifestyle habits have significantly affected parameters investigated cannot be excluded, although, before initiating, all subjects were instructed by physicians and by an expert dietician to maintain their usual lifestyle habits.

Finally, another possible limitation is that a proportion of our samples' subjects were active smokers at the time of enrolment. Cigarette smoking may significantly influence some of the investigated parameters, such as inflammatory variables.

In conclusion, we observed beneficial effects of *cis*-9, *trans*-11 CLA-rich ewe cheese consumption on several risk parameters for atherosclerotic cardiovascular disease.

These observations, although preliminary and obtained in a limited study group, seem to be of relevance for the practical implications in terms of nutrition and health of the general population. If the effects of dairy products naturally enriched for their contents of *cis*-9, *trans*-11 CLA are confirmed by further examinations, this will likely have important implications for human nutrition and food industry.

Conflict of interest

No conflicts of interest are present.

Authors' contribution

FS, AB, RA, MA: study design
FC, AMG, LM, AB, SM: laboratory determinations
FS, AMG: statistical analysis
FS, FC, AB, GFG: writing of the manuscript
RA, LM, GFG, MA: critical revision of the manuscript

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