Journal of Human Hypertension (2012) 26, 127–132

www.nature.com/jhh

ORIGINAL ARTICLE

The effect of conjugated linoleic acid, a natural *trans* fat from milk and meat, on human blood pressure: results from a randomized crossover feeding study

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C*is*-9, *trans*-11 conjugated linoleic acid (CLA) is a natural *trans* fatty acid that is largely restricted to ruminant fats and consumed in foods and supplements. Its role in blood pressure (BP) regulation is still unclear. We examined the effect of c*is*-9, *trans*-11 CLA on BP compared with oleic acid. A total of 61 healthy volunteers were sequentially fed each of 3 diets for 3 weeks, in random order, for a total of 9 weeks. The diets were identical except for 7% of energy (18.9 g in a diet of 10 MJ day^{-1}) that was provided either by oleic acid, by industrial *trans* fatty acids or by *cis*-9, *trans*-11 CLA. We measured BP on two separate days at the end of each intervention period. At baseline, mean BP was 113.8 ± 14.4 mm Hg systolic and 66.3 ± 9.6 mm Hg diastolic. The effect of the CLA diet compared with the oleic acid diet was 0.11 mm Hg (95% confidence interval: −1.27, 1.49) systolic and −0.45 mm Hg (−1.63, 0.73) diastolic. After the industrial *trans* fatty acid diet, the effect was 1.13 mm Hg (−0.25, 2.51) systolic and −0.44 mm Hg (−1.62, 0.73) diastolic compared with the oleic acid diet. Our study suggests that short-term high intakes of *cis*-9,*trans*-11 CLA do not affect BP in healthy volunteers.
Journal of Human Hypertension (2012) **26,** 127–132; doi:10.1038/jhh.2010.132; published online 27 January 2011

Keywords: conjugated linoleic acid; CLA; blood pressure; randomized controlled trial

Introduction

C*is*-9, *trans*-11 conjugated linoleic acid (CLA) is a natural *trans* fatty acid that is produced in the rumens of cows, sheep and other ruminant animals through partial hydrogenation of unsaturated fatty acids from the feed by bacteria. It can also be formed from vaccenic acid in animals and in humans.[1] It is consumed by humans in foods and as supplements. In general, the supplements consist of 50:50 mixtures of the isomers c*is*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA or mixtures of more isomers, whereas CLA in dairy products consists of over 90% of the c*is*-9, *trans*-11 CLA isomer.

CLA has attracted much interest since its favourable effects on health, for example, weight reduction, improved insulin sensitivity and improved blood lipid profiles, have been reported in animal studies.[2] In addition, CLA was found to lower blood pressure (BP) in several rat models.[3–5] On the other hand, CLA is a *trans* fatty acid and its harmful effects on the cardiovascular system cannot be excluded. Little is known about the effect of CLA on human BP. To our best knowledge, only a few BP trials have been done with different mixtures of CLA, all of which had a parallel controlled design. Raff *et al.*[6] examined the effect of a 5-week diet supplemented with 4.7 g of a 50:50 mixture of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA, or a diet low in CLA, on BP and isobaric arterial elasticity in 60 healthy, nonhypertensive young men. Systolic BP nonsignificantly increased by 3 mm Hg in the CLA group compared with the control group (*P*-value: 0.07), whereas diastolic BP did not change. Laso *et al.*[7] studied the effects of a 12-week daily intervention with 3 g of a mixture of multiple CLA isomers or placebo in 60 overweight and obese individuals with a mean BP of 147/84 mm Hg and found no effect on BP. Additionally, Iwata *et al.*[8] did a 12-week trial with a daily intake of 3.4 g CLA mixture in 60 healthy overweight Japanese male volunteers and also found no effect of CLA on BP. Finally, Sluijs *et al.*[9] examined the effect of 6-month daily supplementation with 4 g CLA (2.5 g c*is*-9, *trans*-11 CLA and 0.6 g *trans*-10, *cis*-12 CLA) on

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Received 22 August 2010; revised 16 December 2010; accepted 20 December 2010; published online 27 January 2011

aortic stiffness in 401 overweight and obese adults and found no effect on BP.

We conducted a randomized crossover study to examine the effect of a high dose of c*is*-9, *trans*-11 CLA, from an 80:20 mixture, on BP in 61 normotensive healthy Dutch subjects. The BP effect of CLA was compared with that of industrial *trans* fatty acids and oleic acid, and dietary intakes were fully controlled during the 9-week intervention period.

Subjects and methods

This study formed part of a larger trial that was set up to examine the effect of c*is*-9, *trans*-11 CLA on cardiovascular health.[10] In this study we describe the effect of c*is*-9, *trans*-11 CLA on BP, which was a prespecified secondary end point of the study.

Subjects
The Medical Ethics Committee of Wageningen University approved the study. We recruited men and women aged 18 to 65 years from the Dutch population (Wageningen area) through advertisements. Potential subjects gave written informed consent before screening. We excluded subjects with glycosuria or proteinuria in morning urine samples, and with concentrations of total cholesterol $>6.5\,\mathrm{mmol\,l^{-1}}$ or triglycerides $>2.3\,\mathrm{mmol\,l^{-1}}$. Subjects were also excluded if they suffered from diabetes mellitus or cardiovascular diseases, if they used cholesterol-lowering or antihypertensive medication, had unusual dietary habits including high alcohol intakes, had a body mass index $>30\,\mathrm{kg\,m^{-2}}$ or were pregnant or lactating.

Design and randomization procedure
We performed a randomized multiple crossover controlled feeding trial with three consecutive periods lasting 3 weeks each. The trial ran for 9 weeks in total, from 25 September to 27 November 2007. Before a 2-day run-in period, we randomly assigned subjects to one of the six possible diet sequences by computer-generated numbers. We performed BP measurements and drew blood samples during a pretrial screening visit, on 2 days at the end of each intervention period, and 3 weeks after the end of the study.

Diets and study procedures
All subjects received diets with the same composition but in different amounts depending on their energy requirement. On week days during lunch time, subjects consumed a hot meal at the Division of Human Nutrition of Wageningen University. All other food was provided daily to take away for consumption at home. Foods for the weekend and instructions for its preparation were provided each Friday. The supplied foods provided 90% of each subject's energy requirement. For the remaining 10% of energy, subjects had to select foods from a list of foods and drinks that were low in fat. Subjects recorded the selected foods daily in a diary, as well as any deviations from the diet, illnesses and use of medication. Subjects were asked to maintain their usual lifestyle and were not allowed to drink fortified fruit juices, unfiltered coffee (containing cafestol) or to eat >10 pieces of liquorices a day, as the latter may affect BP. We weighed subjects twice a week and adjusted energy intake when necessary in order to maintain stable body weight.

Experimental fats
The diets were intended to contain the same nutrients except for 7% of total energy, which came from special oils and fats containing c*is*-9, *trans*-11 CLA, industrial *trans* fatty acids or oleic acid. CLA was provided by a CLA-rich oil (donated by Lipid Nutrition, Wormerveer, The Netherlands). Margarines and yogurt drinks enriched with the special oils and fats according to our specifications were manufactured by NIZO Food Research (Ede, The Netherlands). The fat in the oleic acid margarine consisted of 82% high-oleic sunflower oil (Aldoc BV, Schiedam, The Netherlands) and 18% of a standard hard stock, an interesterified mixture of palm oil and palm kernel fat (Unilever Research & Development, Vlaardingen, The Netherlands). The fat in the CLA margarine contained 25% CLA-rich oil, 10% sunflower oil, 47% high-oleic sunflower oil and 18% standard hard stock. The fat of the industrial *trans* margarine consisted of 65% partially hydrogenated vegetable fat (Melano, FUJI Oil Europe, Ghent, Belgium), 10% sunflower oil and 25% high-oleic sunflower oil. In addition, yogurt drinks were produced by enriching fat-free yogurt with 5 g of high-oleic sunflower oil, 5 g of c*is*-9, *trans*-11 CLA-rich oil or 5 g of partially hydrogenated vegetable fat per 100 ml, respectively. The margarines were used as a spread, and as an ingredient in bread, cookies, sauces and gravies. Other food items were bought commercially. Subjects were not informed about the type of diet they consumed until completion of data analysis.

Diet composition
Duplicate diets were analysed for protein, dry matter, ash, dietary fibre and digestible carbohydrates.[11–13] CLA isomers were separated by gas chromatography on a Sil-88 column and other fatty acids on a WAX-58 column (Varian, Middelburg, The Netherlands). The composition of the free-choice items was calculated using Dutch food composition tables and included in the calculation of the average daily intake of nutrients.

Blood pressure measurements
Four trained staff members measured BP at the study centre during a pretrial screening visit, on days 19 and 21 (period 1), days 40 and 42 (period 2), days 61 and 63 (period 3) and 21 days after the end

of the study. After a 10-min rest, three BP measurements with 2 min intervals were performed on the dominant arm in sitting position, using a validated automatic BP device (Omron HEM-907; Omron Healthcare, Inc, Bannockburn, IL, USA) with an appropriately sized cuff. The first measurement was discarded and the subsequent two measurements were averaged. Measurements were repeated after 2 days and values of both occasions were averaged. All BP measurements were performed between 0700 and 0930 hours at a fixed time using the same device by the same staff member for each individual. Subjects remained blinded toward all BP values until data analysis was completed.

Other measurements

Blood samples were taken during the pretrial screening visit and during the intervention on the same occasions when BP measurements took place. Venous blood was collected by nurses after an overnight fast, and all samples were taken at the same time of the day to minimize within-subject variation. The blood samples were directly processed and stored at $-80\,^{\circ}C$. The samples were analysed for blood lipoproteins, as reported elsewhere.[10]

Compliance to the diet was assessed by checking the diaries and by examining the fatty acid composition of cholesterylesters in plasma. The fatty acids in plasma cholesterylesters were analysed as described previously.[14] The results were combined and expressed as a proportion by weight of all fatty acids detected.

Statistical analysis

Double data entry was performed and discrepancies were solved. Treatment codes were broken after blind data analysis.

Values reported in text and tables are means with s.d. or 95% confidence intervals, unless stated otherwise. Response to treatment was defined as the difference in systolic BP between the diets. Data were analysed using the linear mixed model where homogeneous compound symmetry was selected as the covariance structure. Changes in BP values were tested for treatment, period and carryover effects; the latter one was tested by introducing a period by treatment interaction term. The least significant difference test was used for pairwise comparisons.

All analyses were performed using SPSS version 15.0 (SPSS, Chicago, IL, USA). Differences were considered to be statistically significant when $P<0.05$ (two sided).

Results

Subjects

We enroled 63 subjects in the trial. Two subjects withdrew from the trial; one man after 6 days because of personal reasons and one woman after 20 days because of illness, both not related to the study. Figure 1 shows the number of subjects screened, excluded and randomized. The screening characteristics of the subjects who completed the study are shown in Table 1.

Diets and dietary adherence

Mean daily intakes of energy and nutrients according to chemical analysis of the three experimental diets plus calculations from free-choice items are presented in Table 2. Intake of total energy, protein and carbohydrates were similar on all three diets, as were intake of total fat, dietary fibre and alcohol. The cholesterylesters of all subjects showed that the

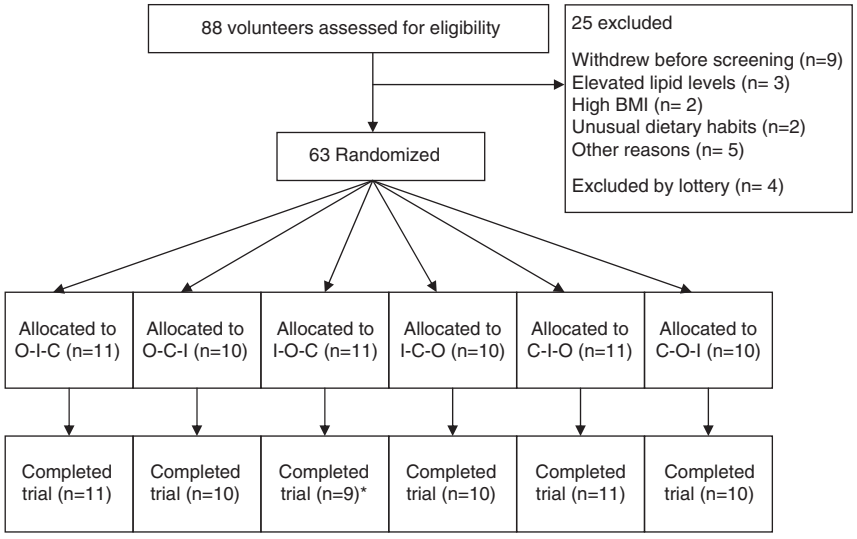

Figure 1 Flowchart of a randomized multiple crossover trial of *cis*-9, *trans*-11 CLA and blood pressure in 61 normotensive Dutch subjects. Each intervention period lasted 3 weeks. Abbreviations: C, *cis*-9, *trans*-11 CLA diet; CLA, conjugated linoleic acid; O, oleic acid diet; I, industrial *trans* fatty acids diet. *Two subjects discontinued the intervention because of personal reasons ($n=1$) and illness ($n=1$) unrelated to the trial. These subjects were not included in the analysis.

Table 1 Baseline characteristics of 61 healthy Dutch subjects who completed the study on CLA and blood pressure

Characteristic	
Age, years	30.9 ± 13.7
Sex, *n* (% male)	25 (41)
Current smokers, *n* (%)	4 (6.6)
BMI, kg m^{-2}	22.8 ± 3.2
Total serum cholesterol, mmol l^{-1}	4.54 ± 0.77
Systolic BP, mm Hg	113.8 ± 14.4
Diastolic BP, mm Hg	66.2 ± 9.6
Heart rate, b.p.m.	73.3 ± 12.4

Abbreviations: BMI, body mass index; BP, blood pressure; CLA, conjugated linoleic acid.
Values are presented as means ± s.d. or numbers (percentages).

CLA proportion was 3.1 times higher after the c*is*-9, *trans*-11 CLA diet than after the oleic acid diet ($P<0.001$).

Diaries kept by the subjects revealed only minor deviations from the protocol. Six subjects reported nausea for 1 or 2 days on the c*is*-9, *trans*-11 CLA and industrial *trans* fatty acids diets, and two subjects while on the oleic acid diet. Three subjects reported diarrhoea for 1 or 2 days while on the c*is*-9, *trans*-11 CLA diet.

Blood pressure

At baseline, mean BP was 113.8 ± 14.4 mm Hg systolic and 66.3 ± 9.6 mm Hg diastolic. Table 3 presents the average BP and heart rate at the end of each dietary intervention period. We observed no significant differences in BP or heart rate among the diets. The effect on BP of the c*is*-9, *trans*-11 CLA diet compared with the oleic acid diet was 0.11 mm Hg (−1.27, 1.49) systolic and −0.45 mm Hg (−1.63, 0.73) diastolic. After the industrial *trans* fatty acids diet, the treatment effect compared with the oleic acid diet was 1.13 mm Hg (−0.25, 2.51) for systolic BP and −0.44 mm Hg (−1.62, 0.73) for diastolic BP. We observed a significant period effect for BP; in the group as a whole, systolic BP increased by 2.38 mm Hg (1.00, 3.75; $P<0.001$). We found no carryover effect for systolic or diastolic BP ($P=0.16$ and $P=0.91$, respectively).

Other measurements

Body weight did not differ among the three diets. Mean body weight (± s.d.) was 70.1 ± 12.2 kg on the oleic acid diet, 70.1 ± 12.2 kg on the industrial *trans* fatty acids diet and 70.1 ± 12.0 kg on the c*is*-9, *trans*-11 CLA diet. However, over 9 weeks of intervention period, the average body weight of the total group decreased by 0.6 ± 1.7 kg ($P=0.006$).

Discussion

In this study among 61 healthy normotensive subjects aged between 18 and 65 years, we found no effect of c*is*-9, *trans*-11 CLA on BP or heart rate. Also, industrial *trans* fatty acids did not affect BP or heart rate.

CLA was found to lower BP in several rat models.[3–5] Therefore, an effect of CLA on BP could be expected in humans as well. A possible mechanism by which CLA and other *trans* fatty acids might influence blood pressure could be through endothelial function. Raff *et al*.[6] investigated the effects of CLA and vaccenic acid on arterial elasticity as a measure of endothelial function. However, they found no difference in effect between CLA and placebo. Recently, Sluijs *et al*.[9] investigated the effects of CLA on aortic stiffness and they also did not show any difference between CLA and placebo treatment.[9] These were both studies using a smaller dose, but a longer duration of supplementation.[6,9] Thus, although the effects of CLA and other *trans* fatty acids on BP via endothelial function could be a logical explanation of the BP effects on animal models, this is not supported by human studies.

The strengths of the study were its randomized crossover design and strictly controlled food intake, which was confirmed by the fatty acid composition of plasma cholesterylesters. We used a strict and standardized protocol for BP measurement (for example, rest, validated device, appropriately sized cuff, blinding). Body weight, an important BP

Table 2 Mean daily intakes of energy and nutrients per diet[a]

	Oleic acid diet	*Industrial* trans *diet*	c*9,t11 CLA diet*
Energy intake, MJ day^{-1}	10.6	10.8	10.7
Kcal day^{-1}	2532	2568	2553
Fat, % of energy	39.7	40.1	39.7
Saturated fatty acids	10.5	13.8	11.3
C12:0 (lauric)	0.7	0.2	0.8
C14:0 (myristic)	0.9	0.7	1.1
C16:0 (palmitic)	5.7	9.7	6.3
C18:0 (stearic)	2.0	2.3	1.9
Total *cis* fatty acids	27.4	17.1	17.7
Cis-9 C18:1 (oleic)	23.1	11.3	13.4
Cis-9, *cis*-12 C18:2 (linoleic)	3.2	4.1	3.4
Total *trans* fatty acids	0.2	7.5	9.1
Total *trans* C18:1	<0.1	7.3	<0.1
Trans-9 C18:1 (elaidic)	<0.1	3.1	<0.1
Trans-10 C18:1	<0.1	1.5	<0.1
Trans-11 C18:1 (vaccenic)	<0.1	0.8	<0.1
Total CLA	0.1	0.1	9.0
Cis-9, *trans*-11 CLA	0.1	0.1	6.9
Trans-10, *cis*-12 CLA	0.0	0.0	1.5
Protein, % of energy	12.6	11.8	12.8
Carbohydrates, % of energy	46.3	46.7	46.0
Alcohol, % of energy	1.4	1.4	1.5
Dietary fibre, g MJ^{-1}	3.0	2.9	2.9
Cholesterol, mg MJ^{-1}	22.3	22.8	21.9

Abbreviations: c9,*t*11, c*is*-9, *trans*-11; CLA, conjugated linoleic acid.
[a]According to chemical analysis of duplicates of the diets (based on three samples per diet); contributions from free-choice items were included as described in Subjects and methods.

Table 3 Blood pressure and heart rate at the end of the three intervention periods in 61 healthy men and women, by treatment

	Oleic acid diet	*Industrial* trans *diet*	c*9,t11 CLA diet*
Systolic BP, mm Hg	113.3 (110.5, 116.1)	112.1 (110.3, 116.0)	113.2 (109.3, 115.0)
Diastolic BP, mm Hg	65.3 (63.3, 67.3)	65.7 (63.7, 67.7)	65.7 (63.7, 67.7)
Heart rate, b.p.m.	70.4 (67.2, 73.6)	70.7 (67.5, 73.9)	70.3 (67.2, 73.5)

Abbreviations: BP, blood pressure; c9,*t*11, c*is*-9, *trans*-11; CLA, conjugated linoleic acid.
Values are mean (95% confidence interval (CI)); data were analyzed using the linear mixed model where homogeneous compound symmetry (CS) was selected as covariance structure. No significant differences among the diets were found.

determinant, was carefully monitored and was similar for the three diets.

The present trial was not primarily designed to investigate the effects on BP, and could therefore have some limitations. Sample size was calculated to be able to detect an effect on low-density lipoprotein cholesterol, the primary outcome of the study. Nevertheless, the power was sufficient to detect small changes in BP: we could have detected a difference in systolic BP of 2 mm Hg with a power of 80%. Second, although food intake was controlled, subjects were free to use coffee and table salt. However, because of the crossover design, the effect of coffee and table salt use is likely to be cancelled out. Furthermore, it should be noted that blinding of treatment was not completely successful. None of the subjects recognized the difference between the c*is*-9, *trans*-11 CLA and the oleic acid diet, but almost all (96%) subjects recognized the industrial *trans* fatty acids diet because of the solid margarine. Yet, as BP was a secondary outcome and most subjects were unaware of this aim of the study, it is unlikely that awareness of the order of the diets has impacted our BP values. Another limitation is the relatively short duration of the dietary intervention periods (3 weeks each). Significant BP changes with dietary measures, however, have been achieved within such a short time period, including the well-known DASH study where most of the treatment effect was already achieved after 2 weeks of dietary intervention.[15] Remarkably, BP of the group as a whole increased slightly but significantly during the 9-week trial (2.4 mm Hg, $P<0.001$). This is in contrast to what is often observed in BP trials. Because BP was a secondary outcome, subjects were not focussed on lowering their BP. Intentional lifestyle or behavioural changes are therefore unlikely and we cannot explain this observation. However, because all subjects received the treatments in random order, we do not think that this has influenced the overall outcome of our study. Finally, our subjects were young, lean and healthy, and the effects that we found in our study may not apply to individuals with higher BP levels, for example, elderly, obese individuals and patients with hypertension.

Up until now, *trans* fatty acids have not been clearly associated with BP.[16–19] However, most previous studies have focussed on industrial *trans* fatty acids[16–19] or CLA mixtures with a high content of *trans*-10, *cis*-12 CLA.[6–8] The *trans*-10, *cis*-12 isomer has been suggested to have more unfavourable effects on cardiovascular health than c*is*-9, *trans*-11 CLA.[2,20] Less was known about the effect of c*is*-9, *trans*-11 CLA. Although different effects of c*is*-9, *trans*-11 CLA on BP might have been expected, the results from our study are in line with previous studies on the effects of industrial *trans* fatty acids and CLA mixtures on BP. Also, the recently published trial by Sluijs *et al*.[9] found no effect of CLA supplementation consisting of predominantly c*is*-9, *trans*-11 CLA on BP or other cardiovascular risk factors.

Thus, we conclude that short-term intake of c*is*-9, *trans*-11 CLA, exclusively found in dairy products, does not affect BP in healthy normotensive human subjects.

What is known about this topic
- C*is*-9, *trans*-11 conjugated linoleic acid (CLA) is a natural *trans* fatty acid that is largely restricted to ruminant fats and consumed in foods and supplements.
- CLA has attracted much interest since its favourable effects on health, for example, weight reduction, improved insulin sensitivity and improved blood lipid profiles, have been reported in animal studies.
- Little is known about the effect of CLA on human blood pressure.

What this study adds
- Short-term high *cis*-9, *trans*-11 CLA intake is unlikely to affect blood pressure in normotensive Dutch subjects.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We are indebted to our dieticians for their assistance during the trial; to all subjects for their enthusiastic participation; to the late Truus Kosmeijer-Schuil for analysis of the fatty acids; and to Dr Eckhard Flöter, Unilever R&D, for technical advice and for delivering the margarine hard stock. This study was

supported by the Netherlands Heart Foundation (Grant no. 2006B176), the Foundation of Nutrition and Health Research and the Royal Netherlands Academy of Arts and Sciences. The Conjugated Linoleic Acid oil was sponsored by Lipid Nutrition, and the margarine hard stock was sponsored by Unilever Research and Development. The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

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