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Effect of α-linolenic acid on 24-h ambulatory blood pressure in untreated high-normal and stage I hypertensive subjects

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Abstract

Results of intervention studies on the effects of α-linolenic acid (ALA; C18 : 3*n*-3) on blood pressure (BP) are conflicting. Discrepancies between studies may be due to differences in study population, as subjects with increased baseline BP levels may be more responsive. Therefore, we examined specifically the effects of ALA on 24-h ambulatory blood pressure (ABP) in (pre-)hypertensive subjects. In a double-blind, randomised, placebo-controlled parallel study, fifty-nine overweight and obese adults (forty males and nineteen females) with (pre-) hypertension (mean age of 60 (SD 8) years) received daily 10 g refined cold-pressed flaxseed oil, providing 4·7 g (approximately 2 % of energy) ALA (*n* 29) or 10 g of high-oleic sunflower oil as control (*n* 30) for 12 weeks. Compliance was excellent as indicated by vial count and plasma phospholipid fatty-acid composition. Compared with control, the changes of –1·4 mmHg in mean arterial pressure (MAP; 24 h ABP) after flaxseed oil intake (95 % CI –4·8, 2·0 mmHg, $P=0·40$) of –1·5 mmHg in systolic BP (95 % CI –6·0, 3·0 mmHg, $P=0·51$) and of –1·4 mmHg in diastolic BP (95 % CI –4·2, 1·4 mmHg, $P=0·31$) were not statistically significant. Also, no effects were found for office BP and for MAP, systolic BP, and diastolic BP when daytime and night-time BP were analysed separately and for night-time dipping. In conclusion, high intake of ALA, about 3–5 times recommended daily intakes, for 12 weeks does not significantly affect BP in subjects with (pre-)hypertension.

Key words: **Human interventions: α-Linolenic acid: Ambulatory blood pressure monitors: Blood pressure**

Hypertension, defined as systolic blood pressure (SBP) >140 mmHg and/or diastolic blood pressure (DBP) >90 mmHg, is a major risk factor for CVD. In fact, hypertension is the leading risk factor contributing to global mortality and affects 40 % of adults worldwide. For this reason, the World Health Organization[1] has identified hypertension as a global public crisis. Early detection and treatment are vital to reduce cardiovascular risk and to prevent cardiovascular events[2]. Therefore, it is important to identify effective strategies to reduce the incidence and prevalence of hypertension, and in this respect, an optimal dietary intake is a proven preventive and supportive strategy.

Evidence suggests that EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3) can lower BP at daily intakes of more than 2 g, with hypertensive individuals being more responsive[3–5]. As EPA and DHA, α-linolenic acid (ALA) is a fatty acid (FA) that belongs to the *n*-3 PUFA family. It is an essential FA mainly derived from plants and found in, for example, nuts, leafy vegetables and plant seed oils such as rapeseed, soyabean and flaxseed oils. Like EPA and DHA, specific dietary guidelines have been formulated for ALA (0·5–1·0 % energy (En%) or about 1–2 g), which are often not met[6]. Although limited, ALA can be converted into EPA and to an even smaller extent into DHA[7]. Like *n*-3 long-chain PUFA, ALA may lower BP[8–11]. However, several other studies found no effects of ALA on BP[12–15]. Noteworthy, none of these trials were performed in subjects with increased BP. In fact, Estruch *et al.*[16] have reported that hypertensive subjects showed significantly larger reductions in SBP than normotensive subjects when given a Mediterranean diet high in ALA. In view of these conflicting findings, we conducted a 12-week, double-blind, randomised parallel, placebo-controlled study to investigate the effects of ALA consumption (4·7 g/d, approximately 2 % of energy intake) on 24-h ambulatory BP (ABP) in a population with high-normal BP or mild hypertension.

Methods

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving humans were approved by the medical ethical committee

Abbreviations: ABP, ambulatory blood pressure; BP, blood pressure; DBP, diastolic blood pressure; FA, fatty acid; HR, heart rate; MAP, mean arterial pressure; SBP, systolic blood pressure.

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of Maastricht University Medical Centre (METC 13-3-064) and registered at ClinicalTrials.gov as NCT02243969. Written informed consent was obtained from all subjects before entering the study.

Study population

Healthy, overweight or obese subjects with a BMI between 25 and 35 kg/m^2 and aged between 40 and 70 years were recruited in and near the vicinity of Maastricht by means of posters in the university and hospital buildings and advertisements in local newspapers. In addition, subjects who had participated in our earlier studies were approached. Potential subjects were invited for two screening visits for the measurement of office BP, height and weight. In addition, a fasting blood sample was taken for analysis of plasma glucose and serum lipid concentrations. Furthermore, subjects were asked to complete a medical and general questionnaire. Eligible for participation were non-smoking men and women with a high-normal BP defined as SBP between 130 and 139 mmHg and/or DBP between 85 and 89 mmHg or stage I hypertension defined as SBP between 140 and 159 mmHg and/or DBP between 90 and 99 mmHg during both screening visits, mean serum total cholesterol:HDL-cholesterol ratio <8, mean serum TAG <4·5 mmol/l and mean plasma glucose <7·0 mmol/l. Subjects with active CVD or severe medical conditions that might interfere with the study outcomes, use of non-steroidal anti-inflammatory drugs, anti-hypertensive, anti-coagulant medication or a diet/medication known to affect serum lipid or glucose metabolism were excluded. Other exclusion criteria were an unstable body weight (weight gain or loss >2 kg in the past 3 months); indication for treatment with medication according to the standard for cardiovascular risk management of the Dutch general practitioners community (Nederlands Huisartsen Genootschap; NHG); drug or alcohol abuse; intense sporting activities >10 h/week; use of nutritional supplements; women expecting changes in the use of oral contraceptives during the study period or lactating, pregnant or intend to become pregnant during study. Finally, subjects had to be willing to give up being a blood donor from 8 weeks before the start of the study and during the study.

Study design

The study had a randomised, double-blind, placebo-controlled parallel design and consisted of a 2-week run-in period followed by a 12-week intervention period. During the entire 14 weeks, subjects were asked to consume daily 5 g of oil at breakfast or lunch and 5 g at dinner. The oils were provided in vials containing 5 g of oil, which were letter coded to blind the subjects and the investigators. During the run-in period, all subjects consumed palm supra olein oil. For the intervention period, they were randomly allocated to either the control or the intervention group, stratified for sex. Subjects in both the groups switched to another treatment oil to maintain blinding. The intervention group received refined cold-pressed flaxseed oil, providing approximately 4·7 g ALA and the control group received high-oleic sunflower oil (Table 1). Subjects were provided with a number of oil vials sufficient for the days until the next visit plus 2 d spare. They were asked to return all unused vials that were counted as a measure of compliance. Although the subjects were free to take the oils according to their own preferences, advice was given to mix the oil with liquid foods such as yogurt, sauces and salad dressing or to use it with bread. It was not allowed to use the oils for baking or frying.

During the 2-week run-in period, subjects visited the research unit at days 0, 11 and 14. The measurements at the end of the run-in period (days 11 and 14) served as baseline measurements. During the 12-week intervention period, subjects visited the university after 6 weeks (day 56) and twice in week 12 (days 95 and 98). At each visit, body weight and office BP were measured, and a fasting blood sample was obtained. In all, 24-h ABP was monitored at the end of the run-in period between days 11 and 14 (baseline) and at the end of the intervention period between days 95 and 98. Food intake over the previous month was assessed at days 14 and 98 by a validated FFQ[17]. Questionnaires were checked by a dietitian, and energy and nutrient intake were calculated using the Dutch food composition table[18]. Subjects were asked to keep their habitual diet, level of physical exercise and alcohol intake throughout the study and to refrain from the consumption of vitamin supplements, capsules providing *n*-3 long-chain PUFA, and products rich in plant stanol or sterol esters three weeks before the start and during the study. Any protocol deviations, signs of illnesses and use of medication or alcohol consumption were recorded in a study diary and was discussed and inspected at each visit. All measurements were performed at the Metabolic Research Unit Maastricht of Maastricht University.

Measurements

Office BP and heart rate (HR) were determined according to the American Heart Association recommendations for BP measurements in humans[19]. Measurements were performed in a seated position after at least 5 min of rest on the left upper arm in fourfold with 1-min intervals using a calibrated Omron device (Omron M7; CEMEX Medische Techniek BV). The first measurement was discarded, and three subsequent measurements were averaged. Mean arterial pressure (MAP) was calculated as $1/3 \times \text{SBP} + 2/3 \times \text{DBP}$, while pulse pressure (PP) was calculated by subtracting the DBP from the SBP.

The 24-h ABP was monitored using an automated BP device (Mobil-O-Graph NG; APC Cardiovascular). BP and HR were measured at 15-min intervals during the day (07.00–23.00 hours) and at 30-min intervals during the night (23.00–07.00 hours). Subjects were asked to maintain their normal daily activities during the recording period and to avoid intense exercise. By pressing a button on the device when going to sleep and waking up, subjects indicated the start of the night and day period. In all, 24-h ABP as well as night-time BP were calculated, which was defined as the average of BP from the time the subjects went to bed until the time they woke up. Daytime BP was defined as the average of BP measurements recorded during the rest of the day. The fall in BP during the night, called night-time dipping, was defined as the difference

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Table 1. Fatty acid (FA) composition of the experimental oils

		Run-in period		Control group		Intervention group	
		Palm supra olein oil		High-oleic sunflower oil		Flaxseed oil	
		Percentage of total FA	g/10 g oil	Percentage of total FA	g/10 g oil	Percentage of total FA	g/10 g oil
PUFA							
C18 : 3 (*n*-3)	α-Linolenic acid	0·2	0·0	–	–	49·4	4·7
C18 : 2 (*n*-6)	Linoleic acid	12·2	1·2	6·3	0·6	15·7	1·5
MUFA							
C18 : 1 (*n*-9)	Oleic acid	47·3	4·5	84·7	8·1	22·2	2·1
SFA							
C18 : 0	Stearic acid	4·2	0·4	3·0	0·3	4·6	0·4
C16 : 0	Palmitic acid	34·1	3·3	3·9	0·4	6·5	0·6
	Other	1·8	0·19	1·9	0·2	1·3	0·1

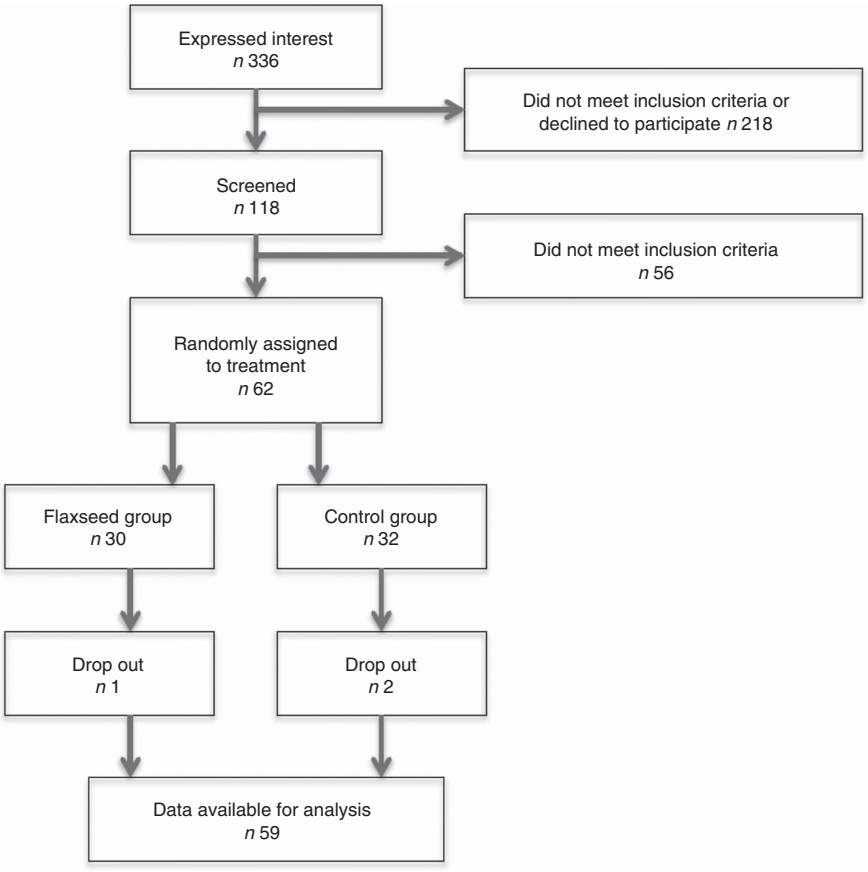

Fig. 1. Subject flow chart of progress through the phases of the study.

between average daytime and average night-time BP expressed as a percentage of the daytime value.

At each visit to the research unit, venous blood was sampled after an overnight fast. On days preceding blood drawings, subjects were asked to avoid the intake of alcohol and not to take part in any strenuous activity. The same person performed each venipuncture at approximately the same time of the day. For the analysis of the FA profile of plasma phospholipids, blood was sampled on days 14 and 95 in EDTA-containing tubes, kept on ice and centrifuged at 1300 ***g*** for 10 min at 4°C without break to obtain plasma. Aliquots were capped under an N flow and stored at –80°C until analysis. After the study, total lipids were first extracted from plasma by means of a modified procedure of the Folch method as described earlier[7,20]. Next, phospholipids were isolated from the total lipid extract on an Extract-Clean NH2-aminopropylsilyl column. FA methyl esters were prepared as described[7,20] and separated and quantified using triple quadrupole GC–MS (Agilent 7000B GC–MS/MS EI system).

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Statistical analysis

Results are presented as means and standard deviations. Where available, individual values of measurements from days 11 and 14 (end of run-in) and of days 95 and 98 (end of intervention) were averaged before statistical analysis. When twenty-five subjects in each group completed the study, the statistical power was more than 80 % to detect a true difference of 3·5 mmHg[11] in mean 24-h ambulatory MAP between the treatments, based on a standard deviation of 8 mmHg, a two-sided α of 0·05 and a correlation coefficient of 0·80 between measurements obtained at the end of the run-in and experimental periods[21]. Data were analysed using a per-protocol approach. Baseline values of the two groups were compared using an unpaired Student's t test. Differences in responses between the flaxseed oil and control groups were evaluated by a one-way ANCOVA with baseline values of the outcome variable as covariate. Differences were considered statistically significant at a two-sided significance level of $P < 0{\cdot}05$. Statistical analyses were performed using SPSS 22.0 for Mac OS X (SPSS Inc.).

Results

Subject characteristics and compliance

Fig. 1 shows the flow of subjects through the study. A total of sixty-two subjects were randomly assigned to either the flaxseed oil group or the control group. In all, one male assigned to the flaxseed oil group dropped out because of a change in medication use. In all, two men assigned to the control group dropped out because of an unexpected change in work and an unplanned holiday. Data from fifty-nine subjects were available for analysis. Inspection of the diaries did not suggest any protocol deviations that may have affected the results. The screening characteristics of these subjects are shown in online Supplementary Table S1. Screening and baseline characteristics of the subjects in the control group were comparable to those in the flaxseed oil group ($P > 0{\cdot}05$ for all variables). Office BP and 24-h ABP were within the range of high-normal BP and stage I hypertension. Compliance was excellent as evidenced from the counts of returned vials, which ranged between 82 and 106 % and was on average 96 %. Apart from intakes of the FA under study, the composition of the diets was similar in both the groups (Table 2). As expected, the flaxseed oil group had a significantly higher intake of total PUFA and ALA, while intake of MUFA, mainly oleic acid, was higher in the control oil group. Body weights did not significantly change during the study and were 88·1 (SD 9·7) kg at the start and 88·5 (SD 9·8) kg at the end for the control group. For the flaxseed oil group, these values were, respectively, 85·3 (SD 10·5) and 86·1 (SD 10·9) kg.

Blood pressure

When compared with the control group, average 24-h ambulatory MAP, SBP, DBP, PP and HR were not significantly affected in the flaxseed oil group (Table 3). Online Supplementary Fig. S1 shows for both the groups the mean hourly ambulatory SBP, DBP and HR as measured for 24 h at baseline and at the end of the intervention period. Separate analyses of daytime and night-time measures of MAP, SBP, DBP, PP and HR did not show differences between treatments. Also, no statistically significant effects were found on night-time dipping of SBP and DBP and on office measurements of MAP, SBP, DBP and HR (Table 3).

Plasma phospholipid fatty acid composition

When compared with the high-oleic control oil, intake of flaxseed oil significantly increased the percentage of total PUFA and total n-3 PUFA in plasma phospholipids (Table 4). More specifically, ALA increased about 2·5-fold by 0·3 % point ($P < 0{\cdot}01$). Also, EPA and DPA significantly increased with 0·6 % point ($P < 0{\cdot}01$) and 0·2 % point ($P < 0{\cdot}01$), respectively. The percentage of DHA and total n-6 PUFA did not change significantly, while the percentage of total MUFA, mainly oleic acid, significantly decreased in the flaxseed oil group when compared with the control group. Also, the percentage of total SFA significantly increased and total *trans*-FA significantly decreased in the flaxseed oil group.

Discussion

In this well-controlled study with untreated (pre-)hypertensive subjects, increasing daily ALA intake to 2·8 En% for 12 weeks, which is about 3–5 times the recommended intake[6], had no effect on BP when compared with the high oleic acid control. Compliance was excellent as indicated by counts of returned vials with the supplemental oils and observed changes in plasma phospholipid FA composition. Other studies that have examined effects of increased ALA consumption on BP have yielded conflicting results. Effects on BP were mainly found in subjects with high-normal BP or hypertension[8,11,22] but not in non-hypertensive subjects[13–15,23]. Therefore, we specifically focused on untreated high-normal and stage I hypertensive subjects. However, our results do not indicate that the discrepant results between the earlier studies can be explained by differences in baseline BP level. Also, the use of anti-hypertensive medication in other studies, which was one of the exclusion criteria in our study, is not likely to be a confounding factor, as decreases in BP with increased ALA have been observed both in studies on subjects with[8,22,24] and without[10,11,25] anti-hypertensive medication. Furthermore, dose and duration cannot be an explanation for the lack of effect on BP in our study. In previous 12-week studies on ALA, reductions in BP have been found with lower daily intakes of ALA – 2·6 g[11] and 3·2 g/d[10] – when compared with the 4·7 g used in our study. In other experiments, the degree of blinding could have influenced the results. Of the positive studies, only the study of Takeuchi *et al.*[11] was double blinded, while other studies were single blinded[10,24] or not blinded at all[8,25]. Of the studies that did not show an effect on BP, the majority was single-[15] or double blinded[13,14,23]; only one study was not blinded[12]. Also, it can be speculated that the FA used to replace ALA intake may have influenced the results. Most of the earlier studies used linoleic acid as control for ALA, while we used oleic acid. However, there are no indications that oleic acid and linoleic acid have differential effects on BP[26]. One

Table 2. Energy and nutrient intake at baseline and after 12 weeks of supplementation with 10 g/d flaxseed oil or high-oleic sunflower oil (HOSF)
(Mean values and standard deviations; mean differences and 95 % confidence intervals)

	Flaxseed group (*n* 29)				Control group (HOSF, *n* 30)						
	Run-in		Intervention		Run-in		Intervention		Treatment effect*		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean difference	95 % CI	*P*
Energy (MJ/d)	10·7	2·2	9·8	1·5	10·3	2·0	10·0	2·2	–0·4	–1·2, 0·3	0·27
Energy (kcal/d)	2555	519	2350	353	2467	483	2396	534	–99·9	–277·8, 78·0	0·27
Protein (En%)	14·8	3·1	14·9	2·4	14·8	2·7	14·8	2·8	0·1	–0·9, 1·1	0·85
Carbohydrates (En%)	40·9	4·6	39·9	5·3	39·9	5·9	39·4	6·5	0·0	–2·8, 2·7	1·00
Total fat (En%)	41·2	4·5	42·1	4·6	42·0	4·4	42·1	5·1	0·4	–1·9, 2·6	0·76
SFA (En%)	13·4	1·9	12·5	2·1	14·2	2·2	13·2	2·6	–0·1	–1·0, 0·8	0·80
MUFA (En%)	15·2	2·8	14·9	3·2	15·7	3·5	17·2	3·2	–1·9	–3·2, –0·6	0·01
Oleic acid (En%)	13·3	2·6	13·0	3·2	13·8	3·0	15·3	3·0	–1·8	–3·1, –0·6	<0·01
PUFA (En%)	8·9	2·5	11·0	2·5	8·2	1·9	7·8	2·3	2·8	1·8, 3·8	<0·01
Linoleic acid (En%)	7·8	2·2	8·1	2·2	7·2	1·7	6·8	2·0	0·9	0·0, 1·8	0·06
α-Linoleic acid (En%)	1·0	0·3	2·8	0·4	0·9	0·2	0·9	0·3	1·9	1·7, 2·1	<0·01
α-Linoleic acid (g/d)	2·8	1·2	7·3	1·0	2·5	0·7	2·3	0·9	4·8	4·4, 5·2	<0·01
EPA (mg)	69	78	68	65	69	89	59	63	9·0	–18·0, 37·0	0·51
DHA (mg)	98	98	100	92	96	123	85	91	14·0	–25·0, 54·0	0·47
trans-Fatty acids (En%)	0·4	0·1	0·4	0·1	0·4	0·1	0·4	0·1	0·0	0·0, 0·1	0·88
Alcohol (En%)	3·0	3·4	3·1	3·5	3·3	2·8	3·7	3·1	–0·3	–0·9, 0·3	0·27
Cholesterol (mg/d)	240	93	228	82	248	89	241	99	–7·2	–38·3, 23·9	0·64
Fibre (g/d)	29	7	26	5	27	8	26	7	–1·2	–4·0, 1·6	0·40

En%, energy percentage.
* Mean difference in change between the flaxseed and control groups with 95 % CI obtained from a one-way ANCOVA with baseline value as covariate.

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Table 3. Ambulatory and office blood pressure measurements at baseline and after 12 weeks of supplementation with 10 g/d flaxseed oil or high-oleic sunflower oil (HOSF) (Mean values and standard deviations; mean differences and 95 % confidence intervals)

	Flaxseed group (*n* 29)				Control group (HOSF, *n* 30)						
	Run-in		Intervention		Run-in		Intervention		Treatment effect*		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean difference	95 % CI	*P*
Ambulatory 24-h blood pressure											
SBP (mmHg)	133·9	7·4	133·9	9·0	135·7	11·1	136·8	13·6	−1·5	−6·0, 3·0	0·51
DBP (mmHg)	85·9	8·2	85·3	8·5	85·3	7·5	86·4	9·3	−1·4	−4·2, 1·4	0·31
MAP (mmHg)	107·9	7·3	107·6	8·1	108·5	8·5	109·5	10·7	−1·4	−4·8, 2·0	0·40
PP (mmHg)	48·1	5·9	48·6	6·6	50·1	7·5	50·3	8·5	−0·1	−2·9, 2·8	0·96
HR (bpm)	70·6	7·6	71·8	8·8	68·2	8·5	68·9	8·3	0·8	−2·2, 3·8	0·59
Night-time dipping SBP (%)	13·7	5·2	13·8	5·8	12·9	5·3	11·0	7·5	2·3	−0·8, 5·4	0·14
Night-time dipping DBP (%)	18·3	6·9	16·5	6·0	16·9	6·0	15·3	7·6	0·6	−2·8, 4·0	0·73
Ambulatory daytime blood pressure											
SBP (mmHg)	138·6	7·7	138·9	10·3	139·9	12·2	140·5	14·4	−0·5	−5·3, 4·3	0·82
DBP (mmHg)	89·9	8·9	89·1	9·4	89·0	7·6	89·6	9·5	−1·3	−4·4, 1·7	0·38
MAP (mmHg)	112·2	7·7	111·9	9·1	112·3	9·0	113·0	11·1	−1·0	−4·7, 2·6	0·58
PP (mmHg)	48·6	6·2	49·8	7·0	50·9	8·4	50·8	9·0	0·8	−2·2, 3·7	0·61
HR (bpm)	73·5	8·0	75·2	10·4	70·2	9·7	71·3	9·2	1·3	−2·4, 4·9	0·49
Ambulatory night-time blood pressure											
SBP (mmHg)	119·4	7·2	119·3	8·0	121·4	8·7	124·6	13·1	−3·7	−8·4, 1·1	0·13
DBP (mmHg)	73·2	7·7	74·1	7·2	73·9	7·9	75·7	9·0	−1·0	−3·9, 1·9	0·50
MAP (mmHg)	94·3	6·9	94·8	6·9	95·7	7·7	98·1	10·3	−2·2	−5·7, 1·3	0·22
PP (mmHg)	46·2	5·5	45·2	6·1	47·5	5·7	49·0	7·8	−2·8	−5·9, 0·3	0·07
HR (bpm)	62·7	7·1	62·4	6·1	61·9	6·2	61·7	6·7	−0·1	−2·4, 2·6	0·94
Office blood pressure											
SBP (mmHg)	142·4	12·3	139·2	9·2	138·6	14·4	138·5	14·5	−1·9	−6·1, 2·3	0·37
DBP (mmHg)	89·6	7·9	88·1	7·6	87·4	7·5	88·0	7·8	−1·8	−3·9, 0·3	0·09
MAP (mmHg)	107·2	8·7	105·1	7·4	104·5	9·0	104·9	9·4	−1·9	−4·5, 0·8	0·17
PP (mmHg)	52·8	8·7	51·1	7·2	51·2	10·7	50·5	10·2	−0·4	−3·4, 2·5	0·77
HR (bpm)	67·8	8·7	68·9	8·4	64·5	9·7	65·0	8·9	1·4	−1·3, 4·1	0·30

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; HR, heart rate; bpm, beats per min.
* Mean difference in change between the flaxseed and control groups with 95 % CI obtained from a one-way ANCOVA with baseline value as covariate.

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Table 4. Fatty acid composition of plasma phospholipids at baseline and after 12 weeks of supplementation with 10 g/d flaxseed oil (*n* 28) or high-oleic sunflower oil (*n* 28)*
(Mean values and standard deviations; mean differences and 95 % confidence intervals)

	Flaxseed group (*n* 28)				Control group (*n* 28)						
	Run-in		Intervention		Run-in		Intervention		Treatment effect†		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean difference	95 % CI	*P*
Total PUFA	39·6	1·8	40·1	1·5	40·0	1·2	39·6	1·3	0·7	0·0, 1·4	0·04
Total *n*-3 fatty acids	5·9	1·4	7·0	0·9	5·6	1·4	5·6	1·6	1·3	0·7, 1·9	<0·01
α-Linolenic acid (C18 : 3*n*-3)	0·2	0·1	0·5	0·2	0·2	0·1	0·2	0·1	0·3	0·2, 0·4	<0·01
EPA (C20 : 5*n*-3)	1·2	0·6	1·7	0·6	1·2	0·7	1·1	0·5	0·6	0·4, 0·9	<0·01
Docosapentaenoic acid (C22 : 5*n*-3)	0·8	0·2	1·0	0·2	0·9	0·2	0·8	0·2	0·2	0·2, 0·3	<0·01
DHA (C22 : 6*n*-3)	3·5	0·9	3·5	0·8	3·2	0·8	3·3	1·1	0·0	–0·4, 0·4	0·97
Total *n*-6 fatty acids	33·6	1·9	33·0	1·5	34·3	1·7	33·9	1·8	–0·6	–1·4, 0·2	0·14
Linoleic acid (C18 : 2*n*-6*cc*)	20·7	2·4	21·0	1·5	20·7	2·0	20·4	2·3	0·7	–0·2, 1·5	0·11
γ-Linolenic acid (C18 : 3*n*-6)	0·1	0·0	0·1	0·0	0·1	0·0	0·1	0·0	–0·0	0·0, 0·0	0·03
Dihomo-*γ*-linolenic acid (C20 : 3*n*-6)	3·3	0·7	2·9	0·6	3·3	0·6	3·4	0·7	–0·4	–0·7, –0·2	<0·01
Arachidonic acid (C20 : 4*n*-6)	8·8	1·3	8·3	1·3	9·3	1·4	9·1	1·4	–0·4	–0·8, 0·0	0·04
Total MUFA	14·6	1·9	13·9	1·9	13·9	1·2	14·7	1·4	–1·4	–2·0, –0·8	<0·01
Oleic acid (C18 : 1*n*-9)	10·6	1·7	9·9	1·8	9·9	1·0	10·6	1·2	–1·2	–1·8, –0·7	<0·01
Total SFA	45·8	1·4	46·1	1·0	46·1	1·0	45·7	1·2	0·5	0·0, 1·0	0·04
Palmitic acid (C16 : 0)	27·7	1·4	27·4	1·4	27·8	1·9	27·5	2·0	0·1	–0·4, 0·5	0·77
Stearic acid (C18 : 0)	13·1	0·8	13·4	0·9	13·3	1·0	13·2	1·0	0·3	0·0, 0·7	0·07
Total *trans*-fatty acids	0·9	0·2	0·9	0·2	0·8	0·2	1·0	0·2	–0·1	–0·2, 0·0	0·01

* Values are expressed as % (w/w) of total fatty acids identified. Only SFA ≥C14 are included.
† Mean difference in change between the flaxseed and control groups with 95 % CI obtained from a one-way ANCOVA with baseline value as covariate.

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other trial reported effects of ALA on BP using oleic acid as control, although in that study hypo-energetic diets were provided[8]. Another possible explanation for discrepant findings between studies could be the source of ALA used. In our study, we used ALA from purified cold-pressed flaxseed oil. Other studies used different sources of ALA, such as mixtures of refined vegetable oils[8,11,15], a specific type of whole grain (Salba)[24] or milled flaxseed[22]. Less purified sources of ALA may contain other bioactive ingredients, such as lignans, peptides, minerals and fibres, that could have cardiometabolic effects[24,27]. However, the effects of these components reported on BP are also not consistent, and sources of ALA in other trials were heterogeneous, and this explanation therefore remains speculative. Taken together, there is no obvious reason that can explain why in our study, unlike in some earlier studies, ALA supplementation did not affect BP. As the design and execution of our study was more rigourous than most of the earlier studies, a conceivable explanation is that ALA intake has no substantial effect on BP and that earlier positive findings were due to chance or potential confounding factors.

A strength of this study is that 24-h ABP measurements were used, which may better predict CVD than office BP[28]. It also allowed us to examine the effects on daytime or night-time BP separately, which were also not significantly affected by flaxseed oil intake. Further, effects on dipping could be calculated, which is the decrease in SBP and DBP during night-time. Subjects in whom the BP reduction during sleep is less pronounced have an increased risk of cardiovascular events and mortality[29]. The observed values in our study could be classified as a normal dipping pattern[29] and remained unaffected during the study.

Analysis of the FA composition of plasma phospholipids not only showed excellent dietary compliance but also showed that the intake of ALA increased EPA and DPA plasma phospholipid levels. This agrees well with results of other studies[20].

In conclusion, we found that higher dietary intakes of ALA, about 3–5 times the recommended daily amounts, for 12 weeks does not significantly affect 24-h ABP or office BP in subjects with (pre-)hypertension.

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D. J. P. and R. P. M. carried out the study and performed the statistical analyses. All authors designed the study, interpreted the data and wrote the manuscript.

D. J. P. and R. P. M. report no conflict of interest. P. L. Z. is and D. F. was employed by Unilever R&D Vlaardingen, Vlaardingen, The Netherlands.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114518003094

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