

Effect of a monounsaturated diet vs. a polyunsaturated fatty acid-enriched diet on blood pressure in normotensive women and men

R. P. MENSINK, A. M. STOLWIJK & M. B. KATAN, Department of Human Nutrition, Agricultural University, Wageningen, The Netherlands

Received 18 September 1989 and in revised form 15 January 1990

Abstract. The effect on blood pressure of monounsaturated and (n-6)polyunsaturated fatty acids was studied under strict dietary control in normotensive subjects. For 17 days 31 women and 27 men received a control diet providing 19.3% of energy as saturated fat. Then subjects were randomized over two test diets: one diet provided 15.1% of energy from monounsaturated and 7.9% from polyunsaturated fatty acids ('mono diet'), and the other diet provided 10.8% from monounsaturated and 12.7% from polyunsaturated fatty acids ('poly diet'). Saturated fat intake was now 12.8% on both diets.

Mean blood pressure at the end of the control period was 116/69 mmHg for the mono group and 117/73 mmHg for the poly group. After 5 weeks on the test diet, blood pressure was 115/67 mmHg for the mono group and 117/72 mmHg for the poly group (difference in changes between the two diet groups was not significant).

These findings suggest that at a high fat intake, linoleic acid, when providing more than 7.9% of energy intake, does not influence blood pressure relative to oleic acid in normotensive women and men.

Keywords. Blood pressure, controlled trial, diet, man, monounsaturated fat, polyunsaturated fat.

Introduction

Elevated levels of blood pressure and of serum total cholesterol are both important causes of premature coronary heart disease [1]. In addition, mild hypertension is a major risk factor for stroke in both men and women [2]. To reduce serum cholesterol levels a reduction in dietary total and saturated fat intake is recommended. The type and amount of dietary fat, however, may also influence blood pressure. Some controlled trials suggested that a cholesterol-lowering linoleic-acid-enriched diet reduces blood pressure,

even in healthy, normotensive subjects [3,4]. Several other studies, however, failed to confirm this [5,6]. On the other hand, epidemiological studies did find a negative association between the percentage of polyunsaturated fatty acids in adipose tissue and blood pressure [7,8]. Even if certain diets rich in (n-6)polyunsaturated fatty acids did lower blood pressure, it is not possible to point out exactly the dietary factor responsible for the hypotensive effect: in addition to an increase in linoleic acid intake, the intake of other nutrients like total fat, potassium, and fibre also changed in the studies discussed above [3,4].

We have tested whether two diets differing in unsaturated fatty acids but not in total fat had an effect on blood pressure in normotensive men and women. Two alternatives to the high-saturated-fat diet that is frequently eaten by affluent populations were compared. One was high and the other moderately high in linoleic acid; the difference between the two diets was made up with oleic acid. The intake of other nutrients was kept constant throughout the study and could therefore not have confounded the results.

Subjects and methods

Subjects

Volunteers were recruited through a local newspaper, through a university newspaper and through posters in university buildings. Subjects were eligible if they (1) had no history of atherosclerotic disease or hypertension, (2) had no anaemia, glycosuria, or proteinuria, (3) were not using any prescribed medicine, (4) were healthy as judged by a physician from a medical questionnaire, and (5) expressed no dislikes for any of the foodstuffs provided during the study. Out of 87 applicants, 83 subjects met these criteria. As we only needed 60 people, we accepted all 28 men and three of their spouses; we then added another 29 women by chance. Nine women used oral contraceptives. Two of the women and one man on the mono diet and two of the women and two of the men on the poly-diet were smokers. Table 1 shows some of their baseline characteristics.

Correspondence: R. P. Mensink, MSc., Department of Human Nutrition, Agricultural University, PO Box 8129, 6700 EV Wageningen, The Netherlands.

Table 1. Age, height, weight, and body mass index (means \pm SD) of the subjects 6 to 4 weeks before the experiment, at the time they were eating their habitual self-chosen diets

	Mono diet		Poly diet	
	Men ($n=14$)	Women ($n=15$)	Men ($n=13$)	Women ($n=16$)
Age (years)	24.3 \pm 7.6	22.9 \pm 3.7	25.9 \pm 6.7	24.4 \pm 6.7
Height (cm)	182.9 \pm 5.3	169.4 \pm 5.3	184.8 \pm 5.5	171.1 \pm 7.4
Weight (kg)	73.1 \pm 7.0	61.6 \pm 5.7	75.1 \pm 7.8	62.3 \pm 7.2
Body mass index (kg m ⁻²)	21.9 \pm 2.3	21.5 \pm 2.1	22.0 \pm 2.1	21.2 \pm 1.4

'Mono' and 'poly' refer to the diet groups into which subjects were randomized during the experiment.

Experimental protocol

The main purpose of the experiment was to compare the effects of a diet rich in monounsaturated fatty acids with a diet rich in polyunsaturated fatty acids on serum lipids. Details of this study have been published [9]. All subjects were studied simultaneously for 55 days from 4 October to 27 November 1987. The first part of the study consisted of a 17-day control period, during which all subjects received a diet high in saturated fatty acids. The subjects were then categorized according to sex, and females also according to use of oral contraceptives. From each category one-half of the subjects chosen at random received a diet rich in monounsaturated fatty acids ('mono diet') for the next 36 days, and the other half a diet rich in (n-6)polyunsaturated fatty acids ('poly diet'). On the mono diet 6.5% of energy from saturated fatty acids was replaced by both monounsaturated and polyunsaturated fatty acids, and on the poly diet by polyunsaturated fatty acids alone. The major difference between both diet groups was the kind of oil used for preparing the food. For the mono diet olive oil was used and for the poly diet sunflower oil. In addition, both diet groups received a diet margarine high in polyunsaturated fatty acids instead of butter. In this way both diets represent practical alternatives for a person who wishes to reduce saturated fat intake. During the study the intake of other nutrients did not change.

Each diet consisted of conventional, mixed solid foods, and menus were changed daily. The amount of food necessary to meet each individual's energy requirement was weighed out. Body weights were checked twice weekly, and energy intake was adjusted when necessary. All food was provided, except for some free-choice items, free from fat and cholesterol. Free-choice items were accorded points corresponding to their energy value, with 1 point equalling 41.8 kJ (10 kcal). Each subject was required to consume a constant number of points per day, which varied with total energy intake and corresponded with 8.9–10.3% of the total energy intake. Typical choices were an apple (6 points), orange juice (5 points) or a glass of beer (8 points). In our experience, the free-choice system helps subjects to reconcile the rigid requirements of participation in a trial with their social life and personal

preferences. The importance of not changing one's selection of free-choice items between periods was repeatedly explained and stressed. Subjects recorded in diaries their free-choice items, the amount of coffee used, and any deviations from the protocol. For each of the three diets, duplicate portions for one imaginary participant with an energy intake of 10 MJ (2390 kcal) per day were collected daily and stored at -20°C for later analysis.

The design and execution of the study was explained to the subjects and informed consent was obtained. Prior approval was obtained from the medical ethical committee of the department. Subjects were asked not to change their smoking habits, use of oral contraceptive, or their physical activity pattern during the study.

Measurements

Before the experiment subjects were asked to weigh and record their food intake on three separate days, including one weekend day. Foods were coded and the composition of the diets calculated using the 1985 edition of the Netherlands Nutrient Data Bank [10] to enable us to estimate each subject's energy and nutrient intake. As it was known from our previous studies that such records tend to underestimate actual energy needs by some 15%, the starting levels of energy intake were set at 15% over stated habitual intake.

Blood pressure was measured with an automatic sphygmomanometer with recorder (Takeda Medical UA-751; Adquipment Medical BV, Rotterdam, The Netherlands). When used in parallel over a period of 3 months, the mean difference between 206 measurements with a standard sphygmomanometer and our automatic device was 4.2 ± 5.1 mmHg for systolic and 2.5 ± 4.3 mmHg for diastolic blood pressure, without any apparent change in time [11]. Blood pressure was measured between 07.30 and 09.30 on two occasions before the study and between 09.00 and 12.30 during weeks 2 to 8 of the experiment. Blood pressure was also measured 11 weeks after the experiment in all subjects, except for one man from each group who were unavailable at that time. Systolic pressure was recorded at Korotkoff phase I and diastolic pressure at Korotkoff phase V. Subjects were asked not to per-

form physical activity or to eat or smoke for 1 hour prior to the blood pressure measurements. Four measurements were made at each session with the subject in the sitting position in a quiet room, after approximately 5 min of rest. Two trained investigators performed the measurements. During the experiment measurements on one subject were always made by the same investigator with the same sphygmomanometer and generally at the same time of the day. In this way, effects of diurnal variation on blood pressure for a subject would cancel out when the difference in blood pressure between the test and control period was calculated.

The fatty acid composition of serum cholesterylesters was determined at the end of the saturated fat period and again at the end of the test period as described [12]. Serum total cholesterol was determined as reported previously [9].

The duplicate portions, as collected daily for an imaginary subject on the control diet, the mono diet and the poly diet, were pooled and mixed thoroughly per diet and then freeze-dried. The ash content and the moisture level [13] were determined and then the material was stored at -20°C . Aliquots were analysed for protein [14] total fat [15], the proportions of individual fatty acids [16], dietary fibre [17], and cholesterol [18]. Available carbohydrate was calculated by difference. Sodium, potassium, calcium and magnesium were determined by atomic absorption spectrophotometry [19] in the freeze-dried material after it had been wet-ashed and neutralized. The mean composition of the diets was calculated from the

duplicate portion analysis plus the calculated contribution of the free-choice items.

Statistical methods

The first blood pressure measurement of each session was discarded and the other measurements were averaged. The response of blood pressure to the mono diet or to the poly diet was calculated for each subject as the change from the end of the control diet period (week 3) to the end of the test period (week 8). Thus each subject was studied on two different diets. Body weights were averaged per week and the change calculated as the difference between week 8 and week 1. An unpaired *t*-test was used to examine differences in effects on blood pressure of the two test diets, relative to the uniform control diet. In addition, blood pressures obtained 11 weeks after the completion of the experiment were compared with week 8, and cholesterol levels with pre-experimental values, using a paired *t*-test. An analysis of variance was carried out to check for possible time effects on blood pressure levels during the test diet period [20]. All *P*-values were two-tailed.

Results

Subjects

One woman and one man withdrew during the first week of the study. The baseline characteristics of the remaining 58 subjects are presented in Table 1.

Table 2. Energy intake and composition of the diets of the subjects before and during the experiment

	Type of diet			
	Habitual* (n=58)	Control† (n=58)	Mono diet‡ (n=29)	Poly diet‡ (n=29)
Energy intake (MJ day ⁻¹)	10.1 ± 2.5	11.7	11.8	11.9
Protein (% energy)	14.3 ± 1.8	13.1	13.4	13.1
Fat (% energy)	35.8 ± 5.6	36.7	37.4	37.6
Saturated fatty acids	14.6 ± 2.9	19.3	12.9	12.6
Monounsaturated fatty acids	12.4 ± 2.2	11.5	15.1	10.8
Polyunsaturated fatty acids	6.3 ± 1.9	4.6	7.9	12.7
P/S ratio‡	0.45 ± 0.2	0.21	0.61	1.00
Carbohydrates (% of energy)	48.6 ± 6.0	49.1	47.8	48.5
Alcohol (% of energy)§	1.7 ± 2.1	1.2	1.5	1.0
Cholesterol (mg MJ ⁻¹)	31.4 ± 12.6	33.4	35.8	35.3
Dietary fibre (g MJ ⁻¹)	3.1 ± 0.9	4.0	4.1	4.1
Sodium (mmol MJ ⁻¹)	12.3 ± 2.3	13.3	12.9	12.5
Potassium (mmol MJ ⁻¹)	9.0 ± 1.9	10.3	10.0	10.2
Calcium (mmol MJ ⁻¹)	3.6 ± 0.9	3.0	3.1	2.9
Magnesium (mmol MJ ⁻¹)		1.5	1.5	1.5

* Values were calculated from 3-day weighed inventories made by the subjects of their habitual food consumption, and they are means ± SD. No figures for magnesium are available in the food composition table used.

† Based on chemical analysis of duplicate diets. SDs for energy intake during the study needed to maintain weight were 2.7 MJ between the subjects on the control diet, 2.8 MJ on the mono diet, and 2.3 MJ on the poly diet.

‡ Ratio of polyunsaturated to saturated fatty acids.

§ During the control diet alcohol intake was 1.3% for subjects who were randomized over the mono diet and 1.1% for those randomized over the poly diet.

Inspection of the diaries showed that in the last 2 weeks of the study, two men and one woman on the mono diet and two men on the poly diet had been sick for 1–3 days. In addition, one man on the mono diet reported that he had not always consumed the prescribed amount of energy from the free-choice items.

Nutrient intake

Table 2 shows that the proportion of energy from protein, fat, carbohydrates and alcohol was essentially the same on the subjects' habitual diets and during the control period. However, the dietary fat composition was different. Saturated fat intake increased from a group average of 14.6% of energy on the habitual diets to 19.3% during the control period, while the intake from polyunsaturates decreased on average by 1.7% of energy. This resulted in a decrease of the P/S-ratio from 0.45 to 0.21. After the switch-over to the two unsaturated fat diets, total fat, alcohol, cholesterol and dietary fibre intake did not change. The intake of saturated fat decreased by 6.5% of energy on both test diets. This decrease was compensated for by an increased intake of monounsaturated (3.5% of energy) and polyunsaturated fatty acids (3.0%) on the oleic-acid-rich diet and by polyunsaturates alone on the linoleic-acid-rich diet. The intake of Na, K, Ca, and Mg was constant throughout the study.

Dietary adherence

Weight changes did not exceed 2.5 kg over the 8 weeks of the experiment, except for one man on the mono-diet who lost 3.2 kg; there was an increase on average of 0.2 ± 1.3 in the mono group and of 0.2 ± 1.0 kg in the poly-group. Pearson's correlation coefficients were calculated between changes in weight and changes in

blood pressure for each diet group and sex separately. They ranged from -0.43 ($P=0.14$) for diastolic pressure in men on the poly diet to 0.37 ($P=0.19$) for systolic pressure in men on the mono diet. Coffee consumption was not different between the two diet groups and amounted on average to 2.6 cups per day on the control diet and on the test diet for the mono group; for the poly group coffee consumption on average was 2.4 cups per day on the control diet and 2.6 cups on the test diet.

Dietary adherence was confirmed by the oleic/linoleic acid ratio of serum cholesterylesters, which decreased from 0.33 ± 0.04 (mean \pm SD) to 0.29 ± 0.05 in the mono group, and from 0.33 ± 0.04 to 0.21 ± 0.03 in the poly group ($P < 0.0001$ for difference in change between the two diet groups).

Serum cholesterol levels of the 56 subjects who were also measured 11 weeks after the experiment, were 4.83 ± 0.69 mmol l^{-1} before the experiment and 4.89 ± 0.78 mmol l^{-1} 11 weeks after the experiment (change: $+0.06 \pm 0.51$ mmol l^{-1} , NS). This suggests that subjects had returned to their habitual dietary fat intake.

Blood pressure

The blood pressure values obtained during the study are shown in Fig. 1. Systolic blood pressure was the same in the two diet groups and remained virtually unchanged during the course of the experiment. Due to higher innate levels, diastolic pressure in the poly group was slightly higher at the end of the control period and it remained so during the test period. During the test period no trend in blood pressure values with time was apparent (data not shown). Mean blood pressures for the two diet groups are shown in Table 3 for men and women separately. Changes in

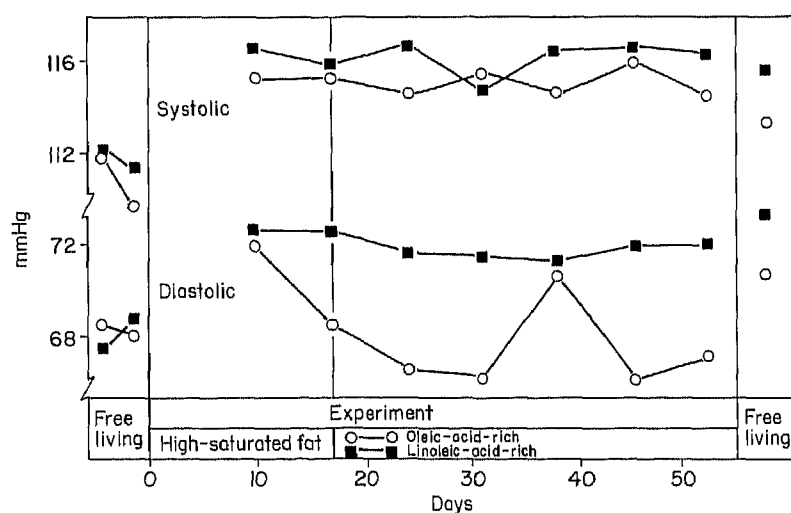


Figure 1. Mean systolic and diastolic blood pressure before, during, and after the experiment. Subjects were volunteers from the general population who were consuming a diet high in saturated fat (week 3) followed by test diets high in either oleic acid (mono group, $n=29$) or in linoleic acid (poly group, $n=29$).

Table 3. Mean systolic and diastolic blood pressure (SD) before, during, and after the experiment (mmHg). Subjects were normotensive volunteers from the general population who were consuming a diet high in saturated fat (week 3) followed by test diets high in either oleic acid (mono group) or in linoleic acid (poly group)

	Mono diet group			Poly diet group		
	Men (n=14)	Women (n=15)	All (n=29)	Men (n=13)	Women (n=16)	All (n=29)
Systolic (mmHg)						
Pre-experimental	119.4 (10.4)	102.5 (7.1)	110.6 (12.2)	118.8 (10.4)	106.2 (11.2)	111.6 (12.4)
Experimental						
Control (week 3)	123.0 (8.3)	108.4 (9.5)	115.5 (11.5)	122.4 (15.1)	111.7 (11.1)	116.5 (13.9)
Test (week 8)	122.6 (10.7)	107.2 (9.2)	114.6 (12.5)	120.0 (9.7)	113.7 (10.9)	116.5 (10.7)
Change	-0.4 (11.0)	-1.2 (7.3)	-0.8 (9.1)	-2.4 (10.2)	+2.0 (7.6)	0.0 (9.0)
Post-experimental*	120.6 (7.7)	107.1 (10.3)	113.4 (11.4)	121.3 (8.8)	112.8 (9.1)	116.4 (9.8)
Diastolic (mmHg)						
Pre-experimental	69.6 (8.3)	67.3 (6.7)	68.4 (7.4)	65.9 (7.8)	69.8 (7.6)	68.1 (7.8)
Experimental						
Control (week 3)	68.8 (7.4)	68.4 (6.9)	68.6 (7.0)	73.5 (9.4)	72.0 (9.0)	72.7 (9.0)
Test (week 8)	68.8 (12.0)	65.5 (6.8)	67.1 (9.6)	69.1 (10.9)	74.4 (8.6)	72.0 (9.8)
Change	+0.1 (7.5)	-2.9† (5.5)	-1.5 (6.6)	-4.4 (9.0)	+2.4 (8.4)	-0.7 (9.2)
Post-experimental*	73.5 (9.0)	68.7 (7.2)	70.9 (8.3)	72.4 (9.7)	74.2 (9.0)	73.4 (9.2)

* One man missing from each diet group.

† Change on mono diet significantly different from change on poly diet: $P < 0.05$.

systolic blood pressure from the saturated to the unsaturated fat diets were slight; they varied from -2.4 ± 10.2 mmHg for men on the poly diet to $+2.0 \pm 7.6$ mmHg for women on the poly diet. The change in diastolic blood pressure of -2.9 mmHg for women on the mono diet was significantly different from that of $+2.4$ mmHg for women on the poly diet ($P = 0.047$, 95% confidence interval for difference in response between the mono group and the poly group: -10.5 to -0.1 mmHg). This effect, however, was not observed in men.

When post-experimental blood pressure levels were compared with those obtained at the end of the experiment, no difference in change between both diet groups was observed.

Excluding the subjects who had fallen ill during the experiment from the analysis ($n = 53$) did not change the results. The change in systolic blood pressure for men on the mono diet ($n = 12$) was now 1.6 ± 10.2 and in diastolic blood pressure 0.7 ± 7.9 mmHg. These changes were not significantly different from those of -1.0 ± 9.1 and -4.7 ± 9.5 mmHg for men on the poly diet ($n = 11$). For women on the mono diet ($n = 14$) the change in systolic blood pressure was -1.1 ± 7.6 compared with 2.0 ± 7.6 mmHg for women on the poly

diet ($n = 16$). The change in diastolic blood pressure of -3.1 ± 5.6 mmHg for women on the mono diet was still greater than that of $+2.4 \pm 8.3$ for women on the poly diet ($P = 0.045$, 95% confidence interval: -10.9 to -0.1 mmHg).

Discussion

In this study with healthy, normotensive subjects we could not demonstrate a favourable effect on blood pressure of linoleic acid, the major essential fatty acid of the (n-6) family, relative to oleic acid when total fat intake was 37%. The effect on diastolic pressure for women was even more favourable on the mono diet than on the poly-diet. This finding, however, may be due to chance, as the direction of the change was opposite to that expected and the effect was not observed in men. The hypothesis to be tested in this experiment was that blood pressure for the two sexes combined would not be affected by linoleic acid relative to oleic acid. This hypothesis could not be rejected. With 26 subjects per diet group the statistical power for detecting a true difference of 4 mmHg between both diets in our study was 52%. To increase the power to 80%, the true difference between both

diets should have been 6 mmHg. It has been shown that a decrease of 7 mmHg in systolic blood pressure is possible in normotensive subjects by dietary modification [21].

The intake of other dietary factors which might influence blood pressure such as sodium, potassium, calcium, magnesium, alcohol, and dietary fibre, was kept constant throughout the study and, therefore, cannot have confounded the results. Changes in weight also cannot have affected blood pressure levels, as they were small and not related to changes in blood pressure. Blood pressure measurements were done automatically to exclude observer bias, and participants did not know the possible changes in blood pressure to be expected. Thus, results were not confounded by other factors known to affect blood pressure.

Our results are in agreement with the results of Sacks *et al.* [22], who also found no effect on blood pressure in normotensive subjects when exchanging oleic acid for linoleic acid. Margetts *et al.* [5] did not observe a change in blood pressure when they increased the dietary P/S ratio from 0.3 to 1.0 by replacing saturated and monounsaturated by polyunsaturated fatty acids. In another study it was shown that blood pressure was not affected if polyunsaturated fat intake increased from 3 to 19% of energy at the expense of a mixture of saturated and monounsaturated fat [6]. Dietary fat intake in these studies [5,6,22] was about 40% of energy; these results suggest that a high fat, linoleic-acid-rich diet has no hypotensive effect in normotensive subjects. Other studies, however, demonstrated a decrease in blood pressure when linoleic acid intake was increased at constant fat intake. Heagerty *et al.* [23] found in a double blind cross-over trial that a supplement of 4 g of safflower oil per day decreased supine systolic pressure in normotensive volunteers. A decrease in both systolic and diastolic blood pressure was reported when the dietary P/S ratio was increased from 0.3 to 1.0, irrespective of whether fat intake was 25 or 43% of energy [24].

A possible explanation for the discrepancy between the present and other studies is that both diets had the same effect on blood pressure. The linoleic acid intake was increased on both diets, and it is possible that the relationship between linoleic acid and blood pressure can only be demonstrated at lower intakes of linoleic acid. However, blood pressure levels did not decrease in either group upon switching from the high-saturated-fat control diet to either unsaturated diet. Although it cannot be ruled out that a downward effect of both unsaturated diets was balanced by an opposite upward drift, this explanation is unlikely; drifts of blood pressure in time are usually downward and not upward in experiments such as ours.

Another explanation for the absence of an effect of linoleate on blood pressure in our study might be attributed to the relatively low initial blood pressure levels of the volunteers. Puska *et al.* [4] indeed found a more pronounced decrease in both systolic and diasto-

lic blood pressure on a low-fat, linoleic-acid-enriched diet in hypertensives than in normotensives. Strazzullo *et al.* [25], however, reported that saturated fat significantly increased systolic pressure in subjects who had a mean systolic pressure that was even lower than that in our sample.

Our results show that two diets differing in the level of unsaturated fatty acids but not in total fat do not influence blood pressure in young normotensive men and women. We conclude that at present there is no conclusive evidence that dietary fat is involved in blood pressure regulation in normotensive men and women.

Acknowledgments

R. P. Mensink is a Dr Dekker fellow of the Netherlands Heart Foundation (grant D-87002). Supported by the Netherlands Nutrition Foundation, and the Netherlands Ministry of Health.

References

- Martin MJ, Hulley SB, Browner WS, Kuller LH, Wentworth D. Serum cholesterol, blood pressure, and mortality: implications from a cohort of 361 662 men. *Lancet* 1986;ii:933-6.
- Dawber TR. The Framingham Study. Cambridge, Massachusetts and London: Harvard University Press. 1980.
- Iacono JM, Marshall MW, Dougherty RM, Wheeler MA, Mackin JF, Canary JJ. Reduction in blood pressure associated with high polyunsaturated fat diets that reduce blood cholesterol in man. *Prev Med* 1975;4:426-43.
- Puska P, Iacono JM, Nissinen A, *et al.* Controlled, randomized trial of the effect of dietary fat on blood pressure. *Lancet* 1983;i:1-5.
- Margetts BM, Beilin LJ, Armstrong BK, *et al.* Blood pressure and dietary polyunsaturated and saturated fats: a controlled trial. *Clin Sci* 1985;69:165-75.
- Brussaard JH, van Raaij JMA, Stasse-Wolthuis M, Katan MB, Hautvast JG AJ. Blood pressure and diet in normotensive volunteers: absence of an effect of dietary fiber, protein, or fat. *Am J Clin Nutr* 1981;34:2023-29.
- Oster P, Arab L, Schellenberg B, Heuck CC, Mordasini R, Schlierf G. Blood pressure and adipose tissue linoleic acid. *Res Exp Med (Berl)* 1979;175:287-91.
- Berry EM, Hirsch J. Does dietary linolenic acid influence blood pressure. *Am J Clin Nutr* 1986;44:336-40.
- Mensink RP, Katan MB. Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. *N Engl J Med* 1989;321:436-41.
- Commissie UCV. UCV Tabel. 's Gravenhage: Voorlichtingsbureau voor de Voeding, 1985.
- van Dusseldorp M, Smits P, Thien T, Katan MB. Effect of decaffeinated versus regular coffee on blood pressure. A 12-week double-blind trial. *Hypertension* 1989;14:563-9.
- Aravanis C, Mensink RP, Karalias N, Christodoulou b, Kafatos A, Katan MB. Serum lipids, apoproteins and nutrient intake in rural Cretan boys consuming high-olive-oil diets. *J Clin Epidemiol*, 1988;41:1123.
- Osborne DR, Voegt P. The Analysis of Nutrients in Foods. London: Academic Press, 1978.
- Williams S, ed. Official Methods of Analysis of the Association of Official Analytical Chemists. 14th ed. Arlington, VA: AOAC Inc, 1984.
- Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957;226:497-509.

- 16 Metcalfe LD, Schmitz AA, Pelka JR. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal Chem* 1966;38:514-15.
- 17 Prosky L, Asp NG, Furda J, de Vries JW, Schweizer TF, Harland BF. Determination of total dietary fiber in foods, food products and total diets: interlaboratory study. *J Assoc Off Anal Chem* 1984;67:1044-53.
- 18 Van de Bovenkamp P, Katan MB. Cholesterol content of chicken skin. *J Food Sci* 1981;46:291.
- 19 Perkin-Elmer Cooperation. *Analytical Methods for Atomic Absorption Spectrophotometry*. Norwalk: Perkin-Elmer Cooperation, 1976.
- 20 Snedecor GW, Cochran WG. *Statistical methods*. 7th ed. Ames: Iowa State University Press. 1980.
- 21 Iacono JM, Puska P, Dougherty RM, *et al*. Effect of dietary fat on blood pressure in a rural Finnish population. *Am J Clin Nutr* 1983;38:860-9.
- 22 Sacks FM, Stampfer MJ, Munoz A, McManus K, Canessa M, Kass EH. Effect of linoleic and oleic acids on blood pressure, blood viscosity, and erythrocyte cation transport. *J Am Coll Nutr* 1987;6:179-85.
- 23 Iacono JM, Judd JT, Marshall MW, *et al*. The role of dietary essential fatty acids and prostaglandins in reducing blood pressure. *Prog Lipid Res* 1981;20:349-64.
- 24 Heagerty AM, Ollerenshaw JD, Robertson DI, Bing RF, Swales JD. Influence of dietary linoleic acid on leucocyte sodium transport and blood pressure. *Br Med J* 1986;293:295-7.
- 25 Strazzullo P, Ferro-Luzzi A, Siani A, *et al*. Changing the Mediterranean diet: effects on blood pressure. *J Hypertension* 1986;4:407-12.