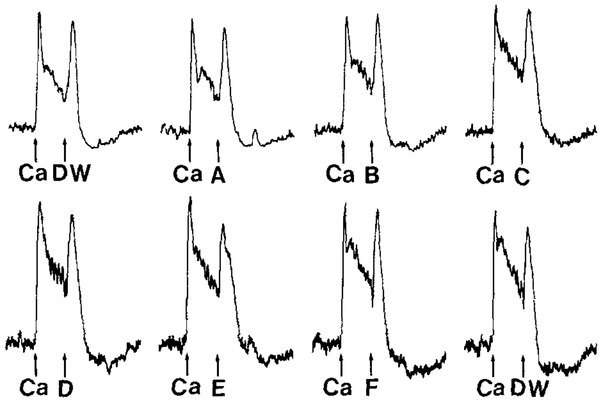

Figure 3. Off-responses produced by rinsing with NaCl of various concentrations (A–F) after 0.01 M $CaCl_2$ (Ca) stimulation for 10 s in a preparation. The magnitude and the temporal pattern of the off-responses do not change appreciably throughout a large concentration range. A, B, C, D, E and F represent 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M, 10^{-3} and 10^{-2} M NaCl, respectively. D.W. = distilled water. Each solution was delivered at the time shown by the arrows with respective letters.

The observation of a reciprocal relation between on- and off-responses for $CaCl_2$, described above, can be explained provided that in each animal the relative ratio of the receptor sites which are stimulated and those which are inactivated by $CaCl_2$ of a given concentration differs, whereas the sum of the receptor sites affected is similar.

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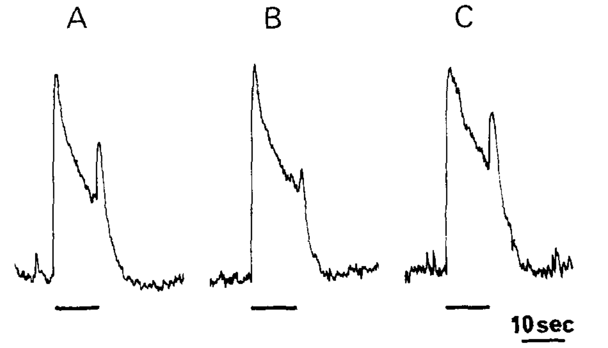

Figure 4. Selective suppression of the integrated off-response after stimulation with $CaCl_2$. On- and off-responses before (A) and after (B) the treatment of the pit organs with 3×10^{-4} M $HgCl_2$ (pH 5.0) for 1 min, and the recovery (C) of the off-response 10 min after the treatment of the organs with 10^{-2} M β-mercaptoethanol (pH 7.5) for 1 min. The bar under each record indicates the duration of the stimulus.

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Blood pressure lowering effect of eicosapentaenoic acid-rich diet in normotensive, hypertensive and hyperlipemic subjects

P. Singer, M. Wirth, W. Gödicke and H. Heine

Zentralinstitut für Herz-Kreislauf-Forschung, Akademie der Wissenschaften der DDR, Wiltbergstrasse 50, DDR-1115 Berlin (German Democratic Republic), 19 April 1984

Summary. A mackerel diet or a herring diet in which two cans of fish fillet were consumed daily over 2 weeks within a prescribed regimen, in a crossover design, were given to 15 normotensive volunteers, 14 patients with mild essential hypertension and eight patients with type IV and V hyperlipoproteinemia (HLP). In normotensives a markedly lower systolic and diastolic blood pressure at the end of the period on the mackerel diet could be observed, whereas in hypertensive and hyperlipemic subjects only systolic blood pressure was significantly decreased. After the herring diet, which served as control, changes in blood pressure were of a minor degree.

Key words. Eicosapentaenoic acid; mackerel diet; blood pressure; essential hypertension; hyperlipoproteinemia.

Several authors have demonstrated that decreased atherogenic serum lipids and lipoproteins as well as prolonged bleeding time and reduced platelet aggregation follows a high intake of n-3 fatty acids in normal and hyperlipemic subjects[1–3]. Recently, a blood pressure lowering effect in normotensive volunteers by a diet supplemented with fish oil[4,5] or canned mackerel[6] was also described.

The data from healthy men suggested that a favorable effect on blood pressure in patients with arterial hypertension and other diseases accepted as risk factors for atherosclerosis might be expected. Therefore, we measured blood pressure in normotensive volunteers and in patients with mild essential hypertension as well as with type IV and V hyperlipoproteinemia (HLP) before and after diets supplemented with canned mackerel and herring, respectively, in a commercially available form, which might be more appropriate to nutritional habits in European populations than supplementation with fish oils[7].

Material and methods. 15 healthy volunteers (10 male, 5 female), 14 male patients with mild essential hypertension (diastolic blood pressure 90–104 mm Hg) and 8 patients (6 male, 2 female) with type IV and V HLP, all receiving no medication, were put on a mackerel diet or a herring diet, for 2 weeks in a crossover design, with a washout interval of 3 months between the dietary periods. Age, b.wt and serum lipids (mean ± SD) of the groups are summarized in table 1.

The diets consisted of 9000–9300 kJ per day with a ratio of carbohydrates:fat:protein of 40:40:20 in percent of kJ, the protocols showing no major deviations from the prescribed regimen. They were supplemented with two cans daily of mackerel or herring fillet, respectively, in tomato pulp. Thus, only the species of fish was changed within the dietary periods. The fat content and fatty acid composition of mackerel, herring and pulp can be seen from tables 2 and 3. The predominant lipids in fish flesh and pulp were triglycerides (table 2). Therefore, only the fatty acid composition of triglycerides in mackerel, herring and tomato pulp is demonstrated (table 3). The percentage of eicosapentaenoic acid (EPA – 20:5, n-3) in mackerel was twice as high and that of docosahexaenoic acid (DHA – 22:6, n-3) three times higher as compared to herring, which served as control. In general, in one half of the subjects the mackerel period preceded the herring period with an interval of 3 months on normal food. With the remaining participants the sequence was reversed. The beginning of the second dietary period corresponded to the control after the first period. The controls after the second period were performed 3 months later.

The total amounts of EPA and DHA ingested were 2.2 g/day and 2.8 g/day, respectively, with the mackerel diet or 1.0 g/day and 1.8 g/day, respectively, during the herring period. The sodium and potassium contents of canned mackerel were slightly higher (0.291 mmoles/g and 0.102 mmoles/g homogenate, respectively) than those of herring (0.231 mmoles/g and 0.090 mmoles/g homogenate, respectively). The sodium intake was free during the dietary periods, the protocols revealing no significant differences between the diets or during the interval on normal food. Sodium and potassium excretion has not been documented in the present experiment, but is included in a further study which is still in progress.

Before and at the end of each dietary period as well as 3 months after the second period blood pressure was measured in triplicate in recumbent and upright position by one observer (P.S.), using the same mercury manometer with a standard cuff (13 × 53 cm) placed about the midpoint of the left upper arm. Diastolic blood pressure was recorded at the disappearance of Korotkoff sounds (phase 5).

Results are given as mean ± SD. Statistical analyses were performed using matched-pair t-test, the levels of significance being accepted as $p < 0.01$ or $p < 0.001$, respectively.

Results and discussion. In normotensive volunteers a significant decrease of systolic and diastolic blood pressure in recumbent position at the end of the mackerel diet could be observed (table 4). Three months later the basal systolic values were not fully reached, whereas diastolic pressure remained significantly lower. No major differences were seen during and after the herring period, although the sodium content of canned herring was slightly lower as compared to mackerel.

In patients with mild essential hypertension only a significant decline of systolic blood pressure after the mackerel diet was confirmed. Again, its return 3 months later did not reach the initial level. Diastolic blood pressure at the end of the mackerel period as well as blood pressure after herring diet were only slightly decreased.

In subjects with HLP a significant fall of systolic blood pressure at the end of the mackerel diet was followed by its significant increase 3 months later. Again, diastolic blood pressure after the mackerel diet and blood pressure after the herring period remained unchanged. In general, no major deviations of blood pressure in the upright position could be found (not demonstrated). The data concerning serum lipoproteins have been published elsewhere in detail[6,7]. In short, serum triglycerides, total and LDL-cholesterol were decreased in all three groups studied, whereas HDL-cholesterol appeared to be elevated at the end of the mackerel, but not after the herring period. This is in agreement with the data from other authors in normal subjects[8,9] and in patients with HLP[10]. For the first time, a lipid lowering effect could also be observed in patients with mild essential hypertension[7]. Both diets were well tolerated without any complaints, and b.wt remained constant during the study.

Table 1. Age (mean ± SD), ideal b.wt, serum triglycerides, total-, LDL- and HDL-cholesterol of the groups studied

	Normotensives (n = 15)	Hypertensives (n = 14)	Hyperlipemics (n = 8)
Age (years)	37.5 ± 5.5	35.3 ± 6.0	44.1 ± 9.9
Ideal weight index (%)	109 ± 9	113 ± 11	113 ± 16
Triglycerides (mmoles/l)	1.23 ± 0.50	1.26 ± 0.42	10.09 ± 10.02
Cholesterol (mmoles/l)	5.26 ± 0.89	5.16 ± 0.80	8.26 ± 2.97
LDL-cholesterol (mmoles/l)	3.31 ± 0.91	3.66 ± 0.94	4.98 ± 1.07 (n = 5)
HDL-cholesterol (mmoles/l)	1.38 ± 0.34	1.25 ± 0.30	1.10 ± 0.23 (n = 5)

Table 2. Content of lipids in mackerel, herring and tomato pulp (g per can)

	Total	Triglycerides	Phospholipids
Mackerel	139	14.3	0.8
Herring	143	12.6	1.1
Tomato pulp	72	12.3	0.2

Table 3. Fatty acid composition of triglycerides in mackerel, herring and tomato pulp

Fatty acids	Mackerel	Herring	Pulp
C_{16}	16.5	14.6	12.1
$C_{18:1}$ (n–9)	22.2	16.2	20.2
$C_{18:2}$ (n–6)	2.9	2.1	7.5
$C_{18:3}$ (n–6)	1.7	0.1	3.8
$C_{18:3}$ (n–3)	2.1	1.2	1.8
$C_{20:1}$ (n–9)	9.5	18.3	9.6
$C_{20:4}$ (n–6)	0.5	0.1	0.4
$C_{20:5}$ (n–3)	4.4	2.1	3.4
$C_{22:1}$*	16.3	25.7	24.7
$C_{22:5}$ (n–3)	0.2	0.1	0.6
$C_{22:6}$ (n–3)	7.0	2.6	4.8

*The docosenoic acids are not subdivided according to the position of their double bond.

Table 4. Systolic (SBP) and diastolic blood pressure (DBP) before and at the end of mackerel and herring diet and after 3 months control in normotensive (n = 15), hypertensive (n = 14) and hyperlipemic subjects (n = 8)

	Before	At the end	After 3 months
Mackerel			
Normotensives-SBP	128 ± 14***	113 ± 11	121 ± 11
-DBP	80 ± 9**	73 ± 10	73 ± 6
Hypertensives-SBP	152 ± 12**	140 ± 11	145 ± 12
-DBP	93 ± 12	89 ± 10	95 ± 11
Hyperlipemics-SBP	144 ± 23**	131 ± 20**	141 ± 21
-DBP	95 ± 13	93 ± 15	95 ± 14
Herring			
Normotensives-SBP	124 ± 12	120 ± 13	124 ± 13
-DBP	75 ± 8	73 ± 6	78 ± 8
Hypertensives-SBP	146 ± 14	137 ± 11	144 ± 11
-DBP	92 ± 10	91 ± 6	94 ± 9
Hyperlipemics-SBP	144 ± 18	140 ± 26	144 ± 22
-DBP	94 ± 16	93 ± 17	98 ± 16

Values are mean ± SD; ***$p < 0.001$, **$p < 0.01$.

The markedly hypotensive effect during the mackerel diet is consistent with the more than twofold higher intake of EPA and DHA with the mackerel as compared to the herring diet. Which of the two n-3 fatty acids is more effective, should be clarified by further studies. The mechanisms involved remain to be elucidated. If the dietary intake of n-3 fatty acids can be confirmed as the determinant factor, blood pressure lowering might be considered to be caused by changes in lipid composition and fluidity of cell membranes at receptor sites of vasoactive hormones or neural transmitters. Furthermore, direct effects of prostaglandins of the 3-series derived from EPA on vessel wall[11-13] or on transmitter release, similar to the effects of prostaglandins of the 2-series[14], should be considered.

From the preventive point of view, it seems relevant that also in patients with essential hypertension and HLP, respectively, a decrease of systolic blood pressure and atherogenic serum lipids by dietary n-3 fatty acids could be confirmed. The results suggest that the favorable influence of diets enriched with n-3 fatty acids on the cardiovascular risk is not restricted to a decrease of atherogenic lipoproteins[8-10], an increase of HDL[15] and reduced platelet aggregation[16,17] but might include other parameters which have not yet been mentioned. Moreover, recent data reveal a beneficial long-term effect of dietary supplementation with fish oils on several risk factors and on coronary heart disease[2]. The data which already exist are encouraging and should stimulate further experimental work.

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An analysis of the distribution of the myelinated nerve fibers in the optic fascicle of a Beagle dog[1]

A. Krinke, E. Fröhlich and G. Krinke

Ciba-Geigy Ltd, CH-4002 Basel (Switzerland), 27 June 1984

Summary. The composition of both optic fascicles of a Beagle dog was studied in topographically oriented, semithin transections of whole nerve stained with toluidine blue. About 165,000 myelinated fibers were present in each nerve, their maximum caliber reaching 11 μm; large, less densely arranged fibers occurred especially in the dorso-temporal region.
Key words. Beagle dog; myelinated fibers; optic fascicle.

Little is known about the composition of the optic nerve in the dog; the cat has been much more studied in this respect. The total number of nerve fibers in the dog optic nerve, estimated by means of a random, sample technique using silver-stained, paraffin sections from ten dogs of various breeds, lies between 126×10^3 and 165×10^3 [2,3]. Our assessment[4] of the number of myelinated fibers in the optic nerve of the beagle dog, using random sampling on toluidine blue-stained, semithin sections from six individuals, indicates an average of 177×10^3.

The technique of random sampling has serious disadvantages, e.g. the sampling error, and the lack of detection of regional differences; for this reason, a complete examination of the whole cross-sectional area of the optic nerve should give optimal results; however, due to the large number of nerve fibers, this approach is only applicable to single specimens.

We report here a total count of the number of myelinated nerve fibers in both optic nerves of a Beagle dog. We have taken into consideration the topographical orientation to show the regional differences in the distribution and caliber of the fibers.

A healthy, purebred, adult, female Beagle dog (five years old and weighing 11.6 kg) was deeply anesthetized with sodium pentobarbital; fixation was immediately carried out by perfusion of 4% formaldehyde followed by 5% glutaraldehyde in 0.1 M phosphate buffer directly into the heart. Portions of the left and right optic fascicles were dissected out; two adjacent transections of each fascicle were taken exactly 8 mm behind the globe (one from each in reserve) after finely notching the dorsal and temporal margins (for the purpose of orientation). The specimens were postfixed with glutaraldehyde, chrome-osmificated with Dalton's solution, and embedded in Spurr epoxy resin. Semithin sections (1 μm) were stained with toluidine blue, and photographed using the light microscope to give a final magnification of × 1000; the whole cross section of the nerve was then reconstructed. A grid was laid on each photomicrograph, subdividing each nerve into square fields, each one representing 40,000 μm² (200 × 200 μm); each field was identified by using a combination of letters and numbers (fig. 1). All myelinated fibers in each square were counted; the average caliber of each fibre was determined in selected fields by measuring the greatest and smallest outer diameter of each myelin sheath.

The right and the left optic nerve both contained a similar number of myelinated nerve fibers (165,238 and 164,888, respectively) despite different sizes in cross-sectional area (2.5092 and 2.8790 mm², respectively); since the smaller right nerve possessed essentially the same number of myelinated fibers as the larger left nerve, its average density of fibers/mm² was higher (65,853