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AN EPIDEMIOLOGICAL AND AN EXPERIMENTAL STUDY ON THE EFFECT OF OLIVE OIL ON TOTAL SERUM AND HDL CHOLESTEROL IN HEALTHY VOLUNTEERS

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We have studied the effects of olive oil on serum lipids in a dietary experiment with 48 healthy adult Dutch volunteers and in 76 boys 8–10 years of age from rural Crete.

The dietary trial was carried out with normolipidaemic subjects, and it compared the effects of an olive-oil-rich and a carbohydrate-rich, high-fibre diet on serum lipids. Replacement of 12% of energy (en%) from saturated fatty acids by either monounsaturates or complex carbohydrates caused the same fall in serum total cholesterol of 0.45 mmol l^{-1}. HDL cholesterol levels fell by 0.19 mmol l^{-1} on the carbohydrate-rich diet, but remained virtually unchanged on the olive-oil-rich diet. This was in agreement with our hypothesis that the total amount of fat in the diet is a determinant of HDL cholesterol.

The diet of the Cretan boys contained 10 en% saturated fat, but 27 en% monounsaturated fat due to the liberal use of olive oil. Serum-lipid levels of the Cretan boys were not different from those of their counterparts from western European Countries. The mean body mass index of the Cretan boys was about 2 kg m^{-2} higher than those of boys in the other countries, which might have confounded comparisons.

Whatever the explanation, the present-day lifestyle and diet in Crete do not produce a particularly favourable HDL to LDL cholesterol ratio in the blood, even though olive oil intake is high.

The Seven Countries Study showed that the incidence of coronary heart disease (CHD) in Crete was lower than would be expected from their total serum cholesterol levels (Keys, *et al.*, 1980). Other risk factors for CHD, such as smoking and hypertension could not explain this observation. Cholesterol in the high density lipoprotein (HDL) fraction, however, was not measured. HDL may protect against CHD (Castelli, *et al.*, 1983).

Reducing serum-cholesterol levels by replacing saturated fat in the diet by carbohydrates lowers not only LDL but, unfortunately, also HDL (Grundy, 1986). Katan (1984) suggested that high-fat diets result in high HDL cholesterol levels, irrespective of the dietary fatty acid composition. An olive-oil-rich diet, which combines a high intake of total fat with a low intake of saturated fat, should therefore lower total serum cholesterol, but not HDL cholesterol. This hypothesis was tested in a strictly controlled dietary intervention study with healthy normolipidaemic volunteers (Mensink & Katan, 1987). In addition, serum lipid values of rural Cretan boys were compared with those of boys from other populations in order to study the long-term effects of olive oil consumption (Aravanis, *et al.*, 1988b).

Methods

Dietary trial

Forty-eight healthy Dutch men and women first consumed a control diet, high in saturated fat for 17 days. The high

intake of saturated fat was mainly achieved by using butter and full-fat dairy products. Then two groups were formed, matched for total and HDL cholesterol, triglycerides (as measured on day 1) and sex. Each group consisted of 12 men and 12 women, three of whom used oral contraceptives. One group received a high-carbohydrate, high-fibre diet and the other group an olive-oil-rich diet for the next 36 days. The composition of the diets is given in Table 1. Subjects on both test diets received low-fat dairy products and subjects on the high-carbohydrate diet were given low-fat margarine. In addition, the high-carbohydrate diet was enriched in bread, pulses, vegetables, potatoes, fruits and jam. Each diet consisted of conventional mixed solid foods, and menus were changed daily. All foodstuffs were supplied individually so as to match each person's energy need. During the study changes in weight did not exceed 2.5 kg.

Fasting blood samples were obtained on days 1, 14, 17 (control period) and on days 30, 46, 49, and 53 (test period). The response to the carbohydrate-rich or the olive-oil-rich diet was calculated per subject as the change from the end of the control-diet (mean of days 14 and 17) to the end of the test period (mean of days, 46, 49 and 53). A unpaired t-test was used to examine the effect of diet on the differences in responses.

Epidemiological study

This study was conducted in cooperation with Professor C. Aravanis, Dr N. Karalias, Professor A. Kafatos and Mrs B. Christodoulou from Greece. Fasting blood samples were drawn from 76 healthy boys from small rural villages in the central part of Crete, south-east of Iraklion, according to the protocol used in earlier studies from Wageningen (Knuiman, *et al.*, 1983). Height and weight of each boy were also measured. After separation of the serum from the blood cells, sera were stored at −20 °C until shipment in the frozen state by air express to the Netherlands, where they were analysed in Wageningen for total and HDL cholesterol, and for triglycerides. In addition, after random pooling of equal amounts of serum from seven to eight boys, cholesterol and triglycerides concentrations were assessed in separate lipoproteins from the twelve pools. Sera of 21 boys, who were not fasting at the time of blood sampling, were

Table 1. *Mean composition of the diets according to duplicate portion analysis.*

	Control period	*Test period*	
	High-saturated fat diet (n = 48)	*Carbohydrate rich diet* (n = 24)	*Olive-oil-rich diet* (n = 24)
Energy intake (MJ)	11.1	11.8	11.1
Protein (% of energy)	13.6	14.1	12.2
Fat (% of energy)			
–Total	38.0	22.1	40.6
–Saturated fatty acids	20.0	6.7	9.8
–Monounsaturated fatty acids	12.4	9.3	24.0
–Polyunsaturated fatty acids	4.1	5.2	5.1
Carbohydrates (% of energy)			
–Total	47.7	62.2	46.0
–Mono- and disaccharides	25.9	32.4	22.4
–Polysaccharides	21.8	29.8	23.6
Alcohol (% of energy)	1.3	1.6	1.2
Cholesterol (mg/day)*	390	390	345
Dietary fibre (g/day)*	42	60	43

*For a subject of average energy intake

included in the pools, because of logistic reasons. Lipoproteins were fractionated by density ultracentrifugation (Terpstra, Woodward & Sanchez-Muniz, 1981). The following density classes (d in g ml^{-1}) were isolated: VLDL ($d < 1.010$), IDL ($1.010 < d < 1.019$), LDL ($1.019 < d < 1.055$), HDL_1 and Lp(a) ($1.055 < d < 1.075$), HDL_2 ($1.075 < d < 1.100$), HDL_3 ($1.100 < d < 1.180$) and a bottom fraction ($d > 1.180$).

Food consumption of each boy was estimated on two consecutive days. Mrs B. Christodoulou met with the mothers and explained how to record the food intake of the children and provided diet scales. Foods were coded and nutrients calculated at the University of Crete, using the USDA food composition table adapted for local use. For foods which were consumed regularly by the boys and for which no nutrient data were available, duplicates were collected on the spot and analysed in Wageningen, and the results were incorporated into the food composition table.

Results

Dietary trial (Mensink & Katan, 1987)
Total serum cholesterol decreased by 0.44 mmol l^{-1} on the high-carbohydrate, high-fibre diet, and by 0.46 mmol l^{-1} on the olive-oil-rich diet (Table 2). HDL cholesterol fell by 0.19 mmol l^{-1} in the carbohydrate group and increased by 0.03 mmol l^{-1} in the olive oil group ($p < 0.001$ for difference in change between both diet groups).

Epidemiological study (Aravanis, *et al.*, 1988b)
Table 3 shows some physical characteristics and serum lipid values of the Cretan boys. None of the boys reported alcohol use or smoking. Table 4 indicates that the intake of saturated fat was relatively low (10 en%), and that of oleic acid high (27 en%), due to the liberal consumption of olive oil. Table 5 shows the distribution of cholesterol and triglycerides over the various lipoproteins.

Table 2. *Effects of a high-carbohydrate, high-fibre diet and an olive-oil-rich diet on serum lipid concentrations (mean ± SD) in men and women.*

	Carbohydrate group	*Olive oil group*
Cholesterol (mmol l^{-1})		
—Control period	5.10 ± 0.95	5.05 ± 0.72
—Test period+	4.66 ± 0.80	4.59 ± 0.68
—Change	−0.44 ± 0.43	−0.46 ± 0.44
HDL cholesterol (mmol l^{-1})		
—Control period	1.42 ± 0.35	1.47 ± 0.39
—Test period	1.23 ± 0.30	1.50 ± 0.34
—Change*	−0.19 ± 0.11	0.03 ± 0.15

Statistical comparison between diets: $*p < 0.001$

Table 3. *Physical characteristics and serum lipid levels of 76 healthy 8–10 year old boys from rural Crete in 1986.*

	Mean	*SD*
Age (months)	108.8	8.5
Height (cm)	133.2	6.2
Weight (kg)	32.5	5.6
Body mass index (kg m^{-2})	18.2	2.4
Total serum cholesterol (mmol l^{-1})	4.42	0.65
HDL cholesterol (mmol l^{-1})	1.40	0.28

Table 4. *Nutrient intakes of 76 healthy 8–10 year old boys from rural Crete in 1986, as determined by two-day recalls*

	Mean	*SD*
Energy (MJ)	11.0	1.9
Protein (% of energy)	10.9	1.8
Carbohydrate (% of energy)	44.3	6.5
Fat (% of energy)		
–Total	45.5	6.2
–Saturated fatty acids	10.0	2.4
–Oleic acid	27.2	4.7
–Linoleic acid	3.4	1.2
Cholesterol (mg/day)	353	192
Fiber (g/day)	18.4	7.8

Table 5. *Cholesterol and triglyceride concentrations in the various lipoproteins in pooled serum samples of healthy 8–10 year old boys from rural Crete in 1986 (n = 12)*

	Cholesterol		*Triglycerides*	
	Mean	*SD*	*Mean*	*SD*
VLDL	0.22	0.11	0.16	0.10
IDL	0.16	0.10	0.04	0.02
LDL	2.12	0.22	0.13	0.02
HDL_1 plus Lp(a)	0.22	0.06	0.01	0.01
HDL_2	0.39	0.06	0.02	0.01
HDL_3	0.94	0.12	0.06	0.01
Bottom fraction	0.15	0.02	0.01	0.00

Discussion

Dietary intervention study

The results of the dietary intervention study showed that the olive-oil-rich diet and the carbohydrate-rich diet lowered total serum cholesterol levels to the same extent, relative to the diet high in saturated fat. This finding agrees with the results of Keys, Anderson & Grande (1957), who concluded from a series of well-controlled studies that replacement of saturated fat by either monounsaturated fat or carbohydrates has the same cholesterol-lowering effect.

HDL cholesterol levels did not change if saturated fat was replaced by monounsaturated fat. On the carbohydrate-rich diet, however, HDL cholesterol fell on average by 0.19 mmol l^{-1}. This observation is in agreement with other studies which showed that total fat intake is a determinant of HDL cholesterol. Becker, *et al.* (1983) compared the effects on serum lipids of three diets, different in fatty acid composition but not in total fat. They found similar HDL cholesterol levels on all three diets, irrespective of the fatty acid composition of the diet. Epidemiological studies have also shown that total fat intake is positively related to HDL cholesterol levels (Knuiman, *et al.*, 1987). There are, however, indications that HDL cholesterol decreases at extreme intakes of PUFA (i.e. 20–30 en%) in the diet (Mattson & Grundy, 1985). Diets providing only 10% of energy as polyunsaturated fat do not lower HDL (Schwandt, Janetschek & Weisweiler, 1982; for a review see Katan, 1984).

Epidemiological study

The results of the epidemiological study are not in agreement with those of the dietary trial. Our hypothesis that Cretan boys would have similar HDL cholesterol levels but lower total serum cholesterol than

boys in other populations was not confirmed. But HDL cholesterol and total serum cholesterol levels were very similar to those in boys from other European populations, where a diet high in saturated and total fat is the norm (Knuiman, *et al.*, 1987). Despite a high olive oil consumption in Crete the mean total serum-cholesterol level (4.42 mmol l^{-1}) was very similar to that of comparable boys in the Netherlands (4.50 mmol l^{-1}), even though the Dutch boys were consuming considerably more saturated fat (10.0 en% in Crete versus 15.1 en% in the Dutch cohort; Knuiman, *et al.*, 1983). According to Key's formula (Keys, *et al.*, 1957), the difference in fatty acid intake should have produced a difference in total serum cholesterol of 0.47 mmol l^{-1}.

A possible explanation for the discrepancy between saturated fat consumption and serum lipid values in our study sample might be their high body mass index. The mean body mass index of the Cretan boys (18.2 kg m^{-2}) was higher than that of comparable boys in other Eurpean countries (15.8–16·8 kg m^{-2}; Knuiman, *et al.*, 1983). In young male adults, body mass index is positively related with serum total cholesterol but negatively with HDL cholesterol (Donahue, *et al.*, 1985). In other words, a beneficial effect of a high olive oil consumption on total serum cholesterol levels might have been undone by the relatively high present-day body mass index in the Cretan boys. Lissner, *et al.* (1987) have shown that a diet high in fat, when compared with a carbohydrate-rich diet, leads to a positive energy balance. Obesity has recently become quite common in Crete (Aravanis, *et al.*, 1988a). It is conceivably due to a decrease in energy expenditure through mechanization of transport and labour. It is, however, unclear whether the difference in fat intake between boys in Crete and boys in some other European countries (46% of energy versus 38–40%) was indeed the cause of the difference in body mass index. It is also uncertain whether the modest elevation of the body mass index in the Cretan boys by itself is enough to undo the expected favourable effect of their low saturated fat intake on total and LDL cholesterol.

Conclusions

We can neither affirm nor reject the hypothesis that under the living and working conditions of the early sixties the Cretan diet and lifestyle produced a favourable serum HDL to LDL cholesterol ratio — we simply came too late to find out. However, under present-day conditions the Cretan diet does not produce a particularly favourable risk profile for CHD.

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