

# Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men<sup>1-3</sup>

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## ABSTRACT

**Background:** Regular consumption of n-3 fatty acids of marine origin can improve serum lipids and reduce cardiovascular risk.

**Objective:** This study aimed to determine whether eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids have differential effects on serum lipids and lipoproteins, glucose, and insulin in humans.

**Design:** In a double-blind, placebo-controlled trial of parallel design, 59 overweight, nonsmoking, mildly hyperlipidemic men were randomly assigned to receive 4 g purified EPA, DHA, or olive oil (placebo) daily while continuing their usual diets for 6 wk.

**Results:** Fifty-six men aged  $48.8 \pm 1.1$  y completed the study. Relative to those in the olive oil group, triacylglycerols fell by  $0.45 \pm 0.15$  mmol/L ( $\approx 20\%$ ;  $P = 0.003$ ) in the DHA group and by  $0.37 \pm 0.14$  mmol/L ( $\approx 18\%$ ;  $P = 0.012$ ) in the EPA group. Neither EPA nor DHA had any effect on total cholesterol. LDL, HDL, and HDL<sub>2</sub> cholesterol were not affected significantly by EPA, but HDL<sub>3</sub> cholesterol decreased significantly (6.7%;  $P = 0.032$ ). Although HDL cholesterol was not significantly increased by DHA (3.1%), HDL<sub>2</sub> cholesterol increased by  $\approx 29\%$  ( $P = 0.004$ ). DHA increased LDL cholesterol by 8% ( $P = 0.019$ ). Adjusted LDL particle size increased by  $0.25 \pm 0.08$  nm ( $P = 0.002$ ) with DHA but not with EPA. EPA supplementation increased plasma and platelet phospholipid EPA but reduced DHA. DHA supplementation increased DHA and EPA in plasma and platelet phospholipids. Both EPA and DHA increased fasting insulin significantly. EPA, but not DHA, tended to increase fasting glucose, but not significantly so.

**Conclusions:** EPA and DHA had differential effects on lipids, fatty acids, and glucose metabolism in overweight men with mild hyperlipidemia. *Am J Clin Nutr* 2000;71:1085-94.

**KEY WORDS** Eicosapentaenoic acid, docosahexaenoic acid, EPA, DHA, hyperlipidemia, fish oil, n-3 fatty acids, lipids, LDL particle size, glucose metabolism, insulin metabolism, men

## INTRODUCTION

There is considerable evidence to support a protective effect of dietary n-3 polyunsaturated fatty acids against atherosclerotic heart disease (1). The 2 principal n-3 fatty acids in marine oils, eicosapentaenoic acid (EPA; 20:5n-3) and docosa-

hexaenoic acid (DHA; 22:6n-3), have a wide range of biological effects (1-3). Those relevant to heart disease include influences on lipoprotein metabolism (4, 5), platelet and endothelial function, vascular reactivity, neutrophil and monocyte cytokine production, coagulation, fibrinolysis, and blood pressure (1-3, 6, 7). In addition, the effect of n-3 fatty acids may be dependent, to some extent, on the presence of underlying disorders such as dyslipidemia, hypertension, diabetes mellitus, and vascular disease.

n-3 Fatty acid supplementation in animals and humans results in substantial increases in plasma and tissue EPA and DHA as well as variable incorporation in different phospholipid classes in different tissues (8-10). These differences may be important to the subsequent utilization and metabolism of EPA and DHA. Although both fatty acids are considered to be biologically active, most studies have focused on the relative importance and effects of EPA, primarily because of its predominance in marine oils and fish species. The recent availability of purified EPA and DHA, however, has enabled studies of the independent biological effects of these fatty acids.

Evidence from in vitro studies suggests differential effects of EPA and DHA (11, 12). In vitro (13) and animal (10, 14, 15) studies have also suggested that EPA may be primarily responsible for the hypotriglyceridemic effect of n-3 fatty acids. Rambjor et al (16) concluded that EPA is responsible for the triacylglycerol-lowering effect of fish oils in humans, but their study had small numbers of subjects and was of short duration. In contrast, a hypotriglyceridemic effect of DHA was shown in healthy subjects (17) and in patients with combined hyperlipidemia (18).

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<sup>2</sup>Supported by a grant (Studies in Hypertension and Cardiovascular Disease) from the National Health and Medical Research Council of Australia. Purified eicosapentaenoic and docosahexaenoic acids and olive oil capsules were kindly provided by the Fish Oil Test Materials Program and the US National Institutes of Health.

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Received August 10, 1999.

Accepted for publication October 20, 1999.

Grimsgaard et al (19) reported that EPA and DHA have similar triacylglycerol-lowering effects compared with corn oil placebo. However, DHA significantly increased HDL cholesterol, whereas EPA significantly lowered both total cholesterol and apolipoprotein (apo) A-I concentrations (19). Neither fatty acid altered LDL-cholesterol concentrations significantly.

Possible benefits of n-3 fatty acids have to be weighed against the potential for impairment of glycemic control, particularly in patients with type 2 diabetes (20–23). However, studies in healthy subjects, in patients with dyslipidemia (24), and in patients with untreated hypertension (25) showed no adverse effects of n-3 fats on plasma glucose concentrations. To our knowledge, there have been no studies in which the effects of pure EPA were compared with those of DHA on indexes of glucose and insulin metabolism in humans.

In view of the increasing use of n-3 fatty acids in the diet as food additives or as therapeutic substances, it is important to determine the extent of any differential effects of EPA and DHA. This study examined the independent effects of EPA and DHA on fatty acid and lipid metabolism, as well as on fasting glucose and insulin concentrations. The study also aimed to determine whether EPA and DHA differ in their effects on HDL-cholesterol subfractions and LDL particle size.

## SUBJECTS AND METHODS

### Study population

Mildly hypercholesterolemic but otherwise healthy, non-smoking men aged 20–65 y were recruited from the general community by media advertising. Entry criteria included a serum cholesterol concentration >6 mmol/L, a triacylglycerol concentration >1.8 mmol/L, or both; a body mass index (BMI; in kg/m<sup>2</sup>) between 25 and 30; and no recent (previous 3 mo) symptomatic heart disease, diabetes, or liver or renal disease (plasma creatinine >130 μmol/L). None of the subjects were regularly taking nonsteroidal antiinflammatory, antihypertensive, or lipid-lowering drugs or other drugs known to affect lipid metabolism. All of the men had a usual weekly consumption of not more than one fish meal and drank <210 mL ethanol/wk. Fifty-nine of the 136 subjects screened satisfied the entry criteria. The study was approved by the ethics committee of the Royal Perth Hospital and all subjects gave written consent.

### Dietary education and intervention

All subjects maintained their usual diets and alcohol intakes during a 3-wk familiarization period. Baseline measurements were collected and the men were stratified for age and BMI before being randomly assigned to 1 of 3 groups: 4 g daily of EPA, DHA, or olive oil (placebo) capsules for 6 wk. Capsules contained either purified preparations of EPA ethyl ester (≈96%), DHA ethyl ester (≈92%), or olive oil (≈75% oleic acid ethyl ester). All participants were instructed to maintain their usual diets, alcohol intakes, and physical activities, and not to make any changes to their lifestyle throughout the intervention period.

At an initial interview, subjects were given written and verbal instructions by a dietitian on how to keep diet records, with food weighed or measured. The same dietitian monitored the dietary intake of all the volunteers at 2-wk intervals and ensured that usual eating habits were maintained. A 3-d diet record (2 week-

days and 1 weekend day) was completed by the volunteers at baseline and postintervention.

### Lifestyle assessment and anthropometry

Alcohol intakes, physical activities, and any medications taken were monitored every second week during the intervention by using 7-d retrospective diaries. Weight was measured every second week with an electronic scale.

### Serum lipids, glucose, and insulin

Fasting serum lipids, lipoproteins, glucose, and insulin were measured twice at baseline and twice at the end of the intervention. Serum glucose was measured with an automated Technicon Axon Analyzer (Bayer Diagnostics, Sydney, Australia) by using a hexokinase method within 12 h of collection. The assay precision for serum glucose at 4.9 mmol/L was 3.1%. Serum insulin was measured by radioimmunoassay with an automated immunoassay analyzer (Tosoh Corporation, Tokyo). The CV for serum insulin at 21 and 102 pmol/L was 14.0% and 8.0%, respectively. The precision in the range of 234–720 pmol/L was 7.0%.

Serum total cholesterol and triacylglycerols were determined enzymatically on the Cobas MIRA analyzer (Roche Diagnostics, Basel, Switzerland) with reagents from Trace Scientific (Melbourne). The assay CVs were 2.2% at 4.2 mmol/L and 1.4% at 10.5 mmol/L for total cholesterol, and 1.6% at 4.0 mmol/L and 2.5% at 1.2 mmol/L for triacylglycerol. HDL cholesterol was determined on a heparin-manganese supernate (26); the CV at 1.1 mmol/L was 1.9%. HDL<sub>2</sub> and HDL<sub>3</sub> cholesterol were determined by using a single precipitation procedure (27). LDL cholesterol was calculated by using the Friedewald formula (28). Serum for the analyses of lipids, lipoproteins, and insulin was snap-frozen in liquid nitrogen and stored at –80°C. Samples obtained at baseline and at the end of the intervention were measured in a single assay to minimize interassay variation.

### LDL particle size

LDL particle size was determined from LDL isolated by vertical density-gradient ultracentrifugation of 4 mL plasma collected into EDTA (29). LDL particle diameter was determined by using a previously published method (30, 31) with use of commercially available 3–13% nondenaturing native gels (Gradipore, Sydney, Australia). Markers used were 28-nm latex beads (Duke, Palo Alto, CA) and high-molecular-weight standards (Pharmacia, Peapack, NJ). Gels were scanned by Tracktel video densitometry (Vision System Ltd, Adelaide, Australia) to provide a quantitative estimate of the dominant peak size. Particle diameter was obtained from a standard curve of the logarithm of the diameter of the standards (latex beads, 28 nm; thyroglobulin, 17 nm; and ferritin, 12.2 nm) against their positions on the scanned gel. A statistical package was used to derive a regression equation that allowed test samples to be sized. The CV of a 26.1-nm quality-control sample run on every gel was 0.8%.

### Plasma and platelet phospholipid fatty acids

Plasma (1 mL) and washed platelets prepared from blood collected into EDTA were extracted with chloroform:methanol (2:1 by vol, 5 mL). The phospholipid fraction was obtained from total lipid extracts by thin-layer chromatography by using a solvent system of hexane:diethyl ether:acetic acid:methanol (170:40:4:4, by vol) on silica gel 60 F<sub>254</sub>-precoated aluminum sheets (Merck, Darmstadt, Germany). Fatty acid methyl esters were prepared by



**TABLE 1**  
Characteristics of participants in the 3 groups at baseline<sup>1</sup>

	Olive oil (control) (n = 20)	EPA (n = 19)	DHA (n = 17)
Age (y)	48.4 ± 2.0	48.9 ± 1.7	49.1 ± 2.2
Body weight (kg)	88.7 ± 2.0	89.1 ± 2.3	90.8 ± 2.8
BMI (kg/m <sup>2</sup> )	28.4 ± 0.5	29.0 ± 0.7	28.9 ± 0.7
Waist-to-hip ratio	0.94 ± 0.01	0.93 ± 0.01	0.94 ± 0.01

<sup>1</sup> $\bar{x} \pm \text{SEM}$ . There were no significant differences by one-way ANOVA. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

treating phospholipid extracts with 4% H<sub>2</sub>SO<sub>4</sub> in methanol at 90°C for 20 min and analyzed by gas-liquid chromatography with a model 5980A gas chromatograph equipped with a 3393A computing integrator (Hewlett-Packard, Rockville, MD). The column was a BPX70 (25 m × 0.32 mm, 0.25- $\mu$ m film thickness; SGE, Ringwood, Australia) with a temperature programmed from 150 to 210°C at 4°C/min and with nitrogen as the carrier gas at a split ratio of 30:1. Peaks were identified by comparing them with a known standard mixture. Individual fatty acids were calculated as a relative percentage with the evaluated fatty acids set at 100%.

### Statistical analysis

Diet records were analyzed by using DIET/1 (version 4; Xyris, Brisbane, Australia), which is based on the Australian Food Composition Database NUTTAB 1995A (32). Data were analyzed by using SPSS (SPSS Inc, Chicago) with general linear models to assess the effects of EPA or DHA relative to the olive oil group. Significance levels were adjusted for multiple comparisons by using the Bonferroni method. Values are reported as means ± SEMs.

## RESULTS

### Study population

Fifty-six of the 59 subjects completed the study. Two subjects withdrew because they were unable to maintain the schedule of laboratory visits and one subject withdrew because of gastrointestinal symptoms. Baseline characteristics of the 3 groups confirmed that they were well matched for the entry criteria (Table 1 and Table 2).

### Energy and macronutrient intakes

Evidence of adherence to the diets was from analysis of diet records and capsule counts. There was no significant difference in body weight between the groups at baseline (Table 1) and no significant change during the intervention. Weight changes in the 3 groups were as follows: 0.2, 0.2, and 0.3 kg in the control, EPA, and DHA groups, respectively. Analysis of diet records indicated that total energy and major macronutrient intakes were not significantly different between groups at baseline (Table 3) and did not change significantly in any of the groups during the intervention. Alcohol drinking and physical activity were unchanged during the intervention in all groups.

### Plasma and platelet phospholipid fatty acids

At baseline, there were no significant differences between groups in plasma and platelet phospholipid fatty acid composi-

tion. The changes in plasma (Figure 1) and platelet (Figure 2) phospholipid fatty acids in each group indicated compliance with capsule intake. There were no significant changes in fatty acid composition in the control group.

#### Plasma fatty acids

In plasma phospholipids, EPA supplementation increased EPA by 494% ( $P < 0.01$ ) and docosapentaenoic acid (DPA; 22:5n-3) by 87% ( $P < 0.01$ ), without significantly changing DHA (9% change; NS). In the DHA group, DHA and EPA increased by 167% ( $P < 0.01$ ) and 52% (NS) respectively, whereas DPA was not affected significantly. Oleic acid (18:1n-9) concentrations were significantly decreased by both EPA (by 11%;  $P < 0.01$ ) and DHA (by 11%;  $P < 0.01$ ) supplementation. There was a significantly larger ( $P < 0.01$ ) decrease in linoleic acid (18:2n-6) in the EPA group (by 21%;  $P < 0.01$ ) than in the DHA group (by 12%;  $P < 0.01$ ). EPA and DHA decreased arachidonic acid (20:4n-6) by 25% ( $P < 0.01$ ) and 22% ( $P < 0.01$ ), respectively, and decreased 20:3n-6 by approximately the same extent, 36% ( $P < 0.01$ ) and 28% ( $P < 0.01$ ), respectively.

#### Platelet fatty acids

EPA supplementation significantly increased platelet phospholipid EPA by 370% ( $P < 0.01$ ) and DPA by 56% ( $P < 0.01$ ), but also significantly decreased DHA by 28% ( $P < 0.01$ ). DHA supplementation significantly increased DHA by 155% ( $P < 0.01$ ) and EPA by 54% (NS). DPA, however, unlike in plasma phospholipids, decreased significantly by 34% ( $P < 0.01$ ). Both EPA and DHA significantly decreased stearic acid (18:0) ( $P < 0.01$ ), whereas only EPA decreased 20:3n-6 (by 25%;  $P < 0.01$ ). Similar to plasma phospholipids, 20:4n-6 decreased significantly more ( $P < 0.01$ ) after EPA (by 15%;  $P < 0.01$ ) than after DHA (by 7%;  $P < 0.01$ ).

### Serum lipids

There were no significant differences in fasting serum lipids at baseline between groups (Table 2). Changes in fasting lipids and lipoproteins are shown in Figures 3 and 4. There were no significant changes in lipids with olive oil supplementation. Neither EPA nor DHA supplementation had an effect on serum total cholesterol concentrations. After adjustment for baseline values, fasting triacylglycerols decreased significantly by 18.4% with EPA ( $P = 0.012$ ) and by 20% with DHA ( $P = 0.003$ ), relative to the placebo group. Serum LDL cholesterol increased significantly with DHA (by 8%;  $P = 0.019$ ), but not with EPA (by 3.5%; NS). In the EPA group, the nonsignificant 3% decrease in HDL cholesterol was attributable to a significant 6.7% reduction in HDL<sub>3</sub> cholesterol ( $P = 0.032$ ) and no change in HDL<sub>2</sub> cholesterol. A small, albeit nonsignificant increase (3.1%) in HDL cholesterol after DHA supplementation was due to a significant increase (29%) in the HDL<sub>2</sub>-cholesterol subfraction ( $P = 0.004$ ) with no significant change in the HDL<sub>3</sub>-cholesterol subfraction.

### LDL particle size

LDL particle size was not significantly different between groups at baseline (Table 2). Neither olive oil nor EPA had a significant effect on LDL particle size, whereas DHA supplementation significantly increased LDL particle size ( $P = 0.002$ ) after adjustment for baseline values (Table 2 and Figure 5). At baseline, LDL particle size was inversely correlated with triacylglycerol ( $r = -0.58$ ,  $P < 0.0001$ ) and positively correlated with

**TABLE 2**Fasting serum lipids, glucose, and insulin at baseline and postintervention in the 3 groups<sup>1</sup>

	Olive oil (control) (n = 20)	EPA (n = 19)	DHA (n = 17)	Treatment effect (P) <sup>2</sup>	
				EPA	DHA
<b>Cholesterol (mmol/L)</b>					
Baseline	6.47 ± 0.21	6.20 ± 0.20	6.18 ± 0.18		
Postintervention	6.22 ± 0.10	6.16 ± 0.11	6.34 ± 0.11	-0.06 ± 0.15 (NS)	0.11 ± 0.15 (NS)
<b>Triacylglycerols (mmol/L)</b>					
Baseline	2.04 ± 0.19	2.01 ± 0.19	2.25 ± 0.40		
Postintervention	1.95 ± 0.10	1.58 ± 0.10	1.50 ± 0.11	-0.37 ± 0.14 (0.012)	-0.45 ± 0.15 (0.003)
<b>LDL cholesterol (mmol/L)</b>					
Baseline	4.41 ± 0.19	4.28 ± 0.19	4.27 ± 0.17		
Postintervention	4.31 ± 0.09	4.46 ± 0.10	4.64 ± 0.10	0.15 ± 0.13 (NS)	0.34 ± 0.14 (0.019)
<b>LDL particle size (nm)</b>					
Baseline	25.68 ± 0.14	25.64 ± 0.09	25.69 ± 0.11		
Postintervention	25.72 ± 0.05	25.69 ± 0.05	25.96 ± 0.06	0.03 ± 0.07 (NS)	0.25 ± 0.08 (0.002)
<b>HDL cholesterol (mmol/L)</b>					
Baseline	1.12 ± 0.07	1.00 ± 0.04	0.96 ± 0.04		
Postintervention	1.02 ± 0.02	0.99 ± 0.02	1.05 ± 0.02	-0.03 ± 0.03 (NS)	0.03 ± 0.03 (NS)
<b>HDL<sub>2</sub> cholesterol (mmol/L)</b>					
Baseline	0.33 ± 0.05	0.25 ± 0.02	0.24 ± 0.03		
Postintervention	0.26 ± 0.02	0.27 ± 0.02	0.33 ± 0.02	0.01 ± 0.02 (NS)	0.07 ± 0.02 (0.004)
<b>HDL<sub>3</sub> cholesterol (mmol/L)</b>					
Baseline	0.80 ± 0.03	0.74 ± 0.03	0.72 ± 0.03		
Postintervention	0.76 ± 0.01	0.72 ± 0.02	0.72 ± 0.02	-0.05 ± 0.02 (0.032)	-0.04 ± 0.02 (NS)
<b>Glucose (mmol/L)</b>					
Baseline	4.95 ± 0.12	5.03 ± 0.09	5.15 ± 0.13		
Postintervention	5.03 ± 0.08	5.24 ± 0.08	5.08 ± 0.09	0.21 ± 0.11 (0.062)	0.05 ± 0.12 (NS)
<b>Insulin (pmol/L)</b>					
Baseline	9.79 ± 1.24	8.78 ± 0.83	9.59 ± 0.99		
Postintervention	8.76 ± 0.51	10.34 ± 0.52	11.38 ± 0.55	1.58 ± 0.73 (0.035)	2.62 ± 0.74 (0.001)

<sup>1</sup> $\bar{x} \pm \text{SEM}$ . There were no significant differences between the 3 groups at baseline (by one-way ANOVA). Postintervention values were not adjusted. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

<sup>2</sup>A general linear model was used to assess treatment effects on postintervention values adjusted for baseline values. Data represent the change and P values for each of the 2 treatment groups relative to the olive oil group.

HDL-cholesterol ( $r = 0.62$ ,  $P < 0.0001$ ) concentrations. The change in LDL particle size from baseline to postintervention was inversely correlated with the change in triacylglycerols ( $r = -0.35$ ,  $P = 0.009$ ) and positively correlated with the change in HDL cholesterol ( $r = 0.28$ ,  $P = 0.035$ ). In regression analysis, DHA supplementation remained the strongest independent predictor of postintervention LDL particle size (adjusted  $R^2 = 0.776$ ,  $P = 0.002$ ) after adjustment for baseline values and for the change in LDL particle size (adjusted  $R^2 = 0.216$ ,  $P = 0.023$ ). DHA supplementation remained significant in regression models, which included changes in serum triacylglycerols and other lipids.

### Glucose and insulin

At baseline there were no significant differences between groups in fasting serum glucose or insulin concentration (Table 2). Postintervention, however, there were significantly different responses between the EPA and DHA groups (Figure 6). Olive oil did not change either fasting glucose or insulin. After

adjustment for baseline values, there was a trend toward increased fasting glucose concentrations with EPA ( $P = 0.062$ ), but not with DHA (NS), relative to the control group. Both EPA and DHA significantly increased fasting insulin, by 18% ( $P = 0.035$ ) and 27% ( $P = 0.001$ ), respectively. DHA supplementation also significantly decreased the glucose-insulin ratio by  $0.13 \pm 0.05$  ( $P = 0.018$ ).

### DISCUSSION

This study addressed whether purified EPA and DHA have different effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. We found that DHA, but not EPA, improved serum lipid status, in particular a small increase in HDL cholesterol and a significant increase in the HDL<sub>2</sub>-cholesterol subfraction, without adverse effects on fasting glucose concentrations. Neither EPA nor DHA affected total cholesterol and both fatty acids reduced



**TABLE 3**

Total energy and macronutrient intakes at baseline and changes during the intervention in the 3 groups<sup>1</sup>

	Olive oil (control) (n = 20)	EPA (n = 19)	DHA (n = 17)
Total energy intake (kJ/d)			
Baseline	10441 ± 588	9516 ± 677	10550 ± 588
Change	-471 ± 497	82 ± 844	-188 ± 421
Total fat (% of energy)			
Baseline	34.2 ± 1.2	30.9 ± 1.6	32.6 ± 1.6
Change	-0.4 ± 1.3	2.8 ± 1.6	2.3 ± 1.2
Fatty acids (% of energy)			
Saturated fat			
Baseline	13.6 ± 0.8	12.1 ± 0.9	13.6 ± 0.9
Change	0.0 ± 0.6	1.2 ± 0.9	0.8 ± 1.0
Monounsaturated fat			
Baseline	12.2 ± 0.6	11.1 ± 0.8	11.5 ± 0.7
Change	-0.2 ± 0.6	1.4 ± 0.8	0.3 ± 0.6
Polyunsaturated fat			
Baseline	5.0 ± 0.3	4.2 ± 0.3	4.6 ± 0.2
Change	0.0 ± 0.4	0.2 ± 0.4	0.9 ± 0.5
Protein (% of energy)			
Baseline	18.0 ± 0.5	19.8 ± 0.9	18.1 ± 0.6
Change	-0.5 ± 0.6	-0.9 ± 0.8	0.1 ± 0.7
Carbohydrate (% of energy)			
Baseline	42.1 ± 1.7	44.6 ± 1.7	41.8 ± 1.8
Change	1.9 ± 1.6	-1.0 ± 1.5	-1.2 ± 1.4
Fiber (g/d)			
Baseline	30.8 ± 2.9	27.4 ± 1.8	26.1 ± 1.1
Change	-3.3 ± 2.3	-2.8 ± 2.1	-0.1 ± 1.7

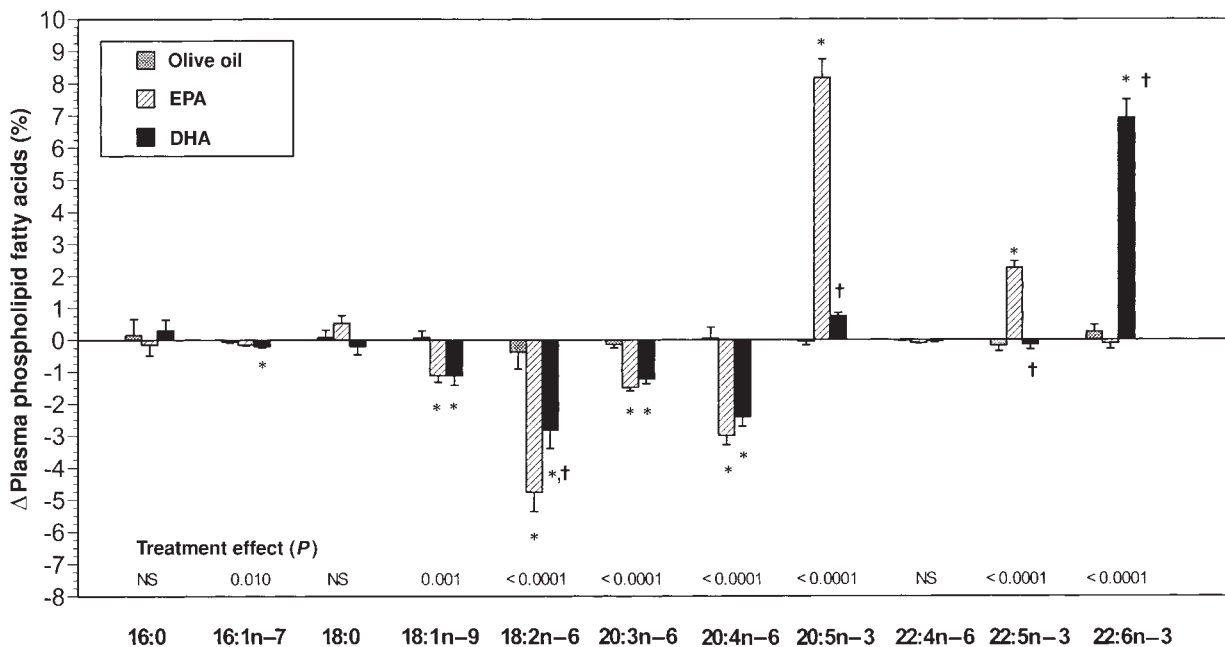
<sup>1</sup> $\bar{x} \pm \text{SEM}$ . Baseline measures were compared by one-way ANOVA. A general linear model was used to test for treatment effects on postintervention values adjusted for baseline value. There were no significant differences between the groups in any of the dietary nutrients at baseline and no significant changes during the intervention. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

triacylglycerols and increased fasting insulin concentrations to a similar extent. DHA supplementation significantly increased LDL cholesterol; however, this was associated with an increase in LDL particle size, which may represent a shift to a less atherogenic LDL particle.

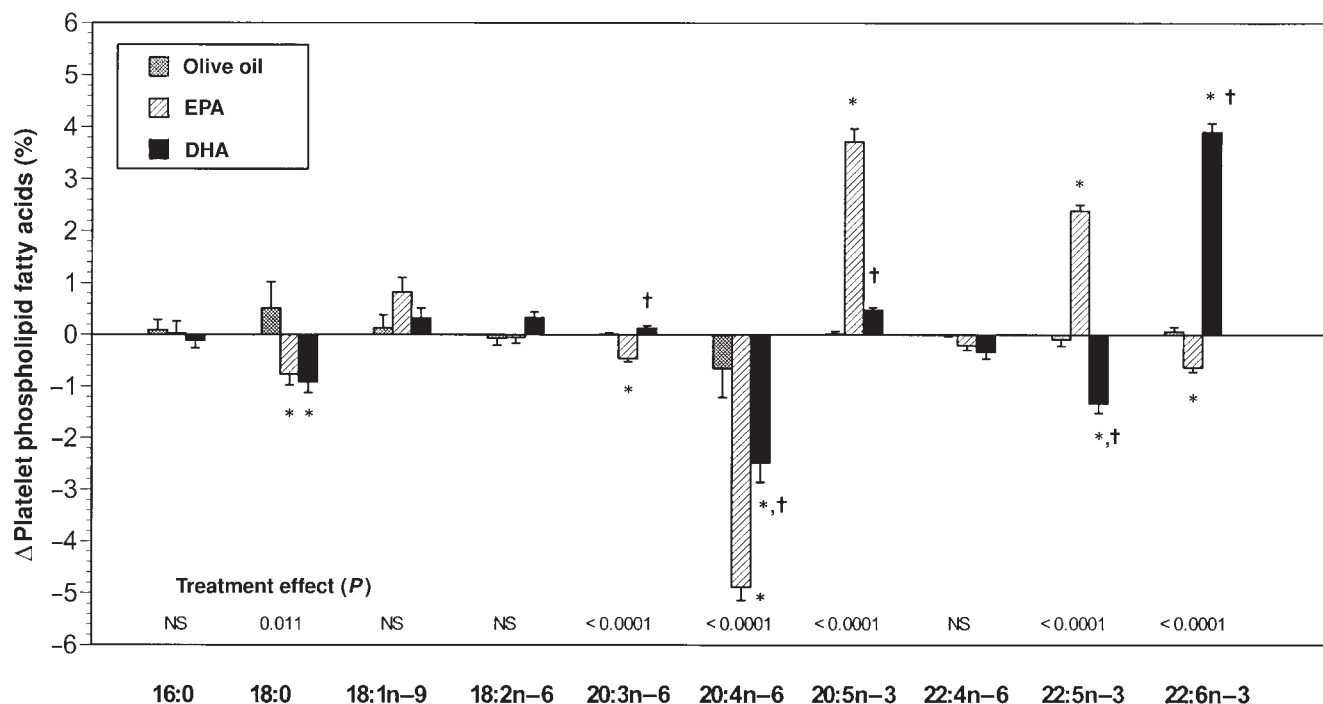
Although numerous studies have examined the effect of n-3 fatty acids on serum lipids, glucose, and insulin (1-5, 20-22), few have assessed the independent effects of EPA and DHA. In vitro, both EPA (13, 33-35) and DHA (13, 35, 36) inhibit triacylglycerol synthesis and secretion. In rats, EPA lowered triacylglycerols, whereas DHA lowered cholesterol (10, 14, 15). These studies, however, used very high doses (1-2 g·kg<sup>-1</sup>·d<sup>-1</sup>) of fatty acids, equalling 12-24 g/d in humans.

In humans, n-3 fatty acids reduce triacylglycerols (4, 5, 37), with more variable effects on total cholesterol, LDL cholesterol, and HDL cholesterol (4, 5). These contradictory findings may be explained, in part, by variations in the amount of n-3 fatty acids consumed, the manner in which they are presented (fish, fish oils, or purified oils), and the lipoprotein phenotype of the patients. Our own studies have shown that the background dietary fat intake influences serum lipid responses to n-3 fatty acids (37).

Trials in humans using mixtures enriched in EPA and DHA have suggested different effects of the 2 fatty acids on serum lipids (38, 39). In a placebo-controlled study, 4 g EPA/d reduced triacylglycerols by 35% (40). It was also shown in a single-blind crossover study that EPA reduced triacylglycerols and VLDL cholesterol, increased LDL cholesterol and HDL cholesterol, but had no effect on total cholesterol (16). DHA did not affect cholesterol, triacylglycerols, VLDL cholesterol, LDL cholesterol, or HDL cholesterol, but increased the HDL<sub>2</sub>-cholesterol subfraction and reduced the HDL<sub>3</sub>-cholesterol subfraction (16). That study, however, had only a small number of subjects in the DHA group, was short in duration, and included only a 2-wk washout period between treatments (16).



**FIGURE 1.** Mean ( $\pm$ SEM) changes in plasma phospholipid fatty acids from baseline to the end of the intervention in the olive oil (control;  $n = 20$ ), eicosapentaenoic acid (EPA;  $n = 19$ ), and docosahexaenoic acid (DHA;  $n = 17$ ) groups. ANOVA was used to assess treatment effects. \*Significantly different from the olive oil group,  $P < 0.01$ . †Significantly different from the EPA group,  $P < 0.01$ .



**FIGURE 2.** Mean ( $\pm$ SEM) changes in platelet phospholipid fatty acids from baseline to the end of the intervention in the olive oil (control;  $n = 20$ ), eicosapentaenoic acid (EPA;  $n = 19$ ), and docosahexaenoic acid (DHA;  $n = 17$ ) groups. ANOVA was used to assess treatment effects. \*Significantly different from the olive oil group,  $P < 0.01$ . †Significantly different from the EPA group,  $P < 0.01$ .

Several reports have described the effects of DHA supplements on serum lipids in humans. Nelson et al (17), in a single-blind study of healthy men, compared the effects of 6 g DHA/d with those of a control diet. They reported that after 90 d total cholesterol, LDL cholesterol, apo A-I, apo B, and lipoprotein(a) were unchanged, whereas triacylglycerols decreased and HDL cholesterol increased. Similarly, in patients with combined hyperlipidemia, Davidson et al (18) compared the effects of 1.25 and 2.5 g DHA/d with those of a vegetable-oil control and showed that DHA reduced triacylglycerols significantly. The higher dose of DHA was also associated with a significant increase in LDL cholesterol.

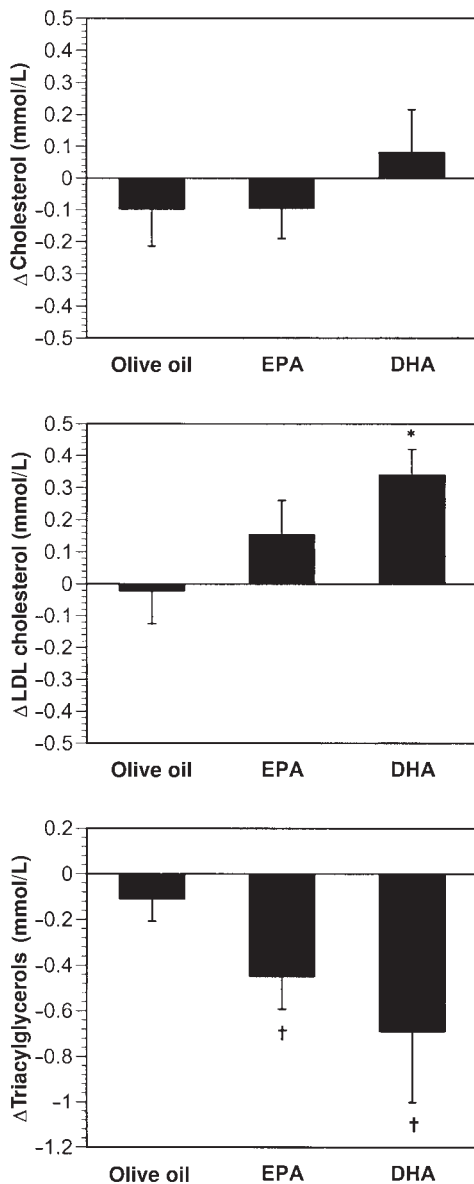
In another study, healthy, nonsmoking men were supplemented daily with 4 g EPA, DHA, or corn oil for 7 wk (19). Both EPA and DHA reduced triacylglycerols, by 21% and 26%, respectively. In the present study, the same dose of EPA and DHA for 6 wk reduced triacylglycerols by 18% and 20%, respectively. We observed no significant effect of EPA or DHA on total cholesterol. In contrast, Grimsgaard et al (19) reported increased total cholesterol with EPA. The difference in results between the 2 studies may have been due to differences in the baseline serum lipid concentrations of the subjects.

It has been suggested that serum HDL cholesterol is better maintained with DHA-enriched than with EPA-enriched oils (38). The present data and previous findings (19) support this hypothesis. We observed that the increase in HDL cholesterol was due to a 29% increase in HDL<sub>2</sub> cholesterol. Increased HDL<sub>2</sub> cholesterol was reported previously by our group after daily consumption of fish or fish oils by subjects with type 2 diabetes or at risk of heart disease (23, 37). In contrast, Grimsgaard et al (19) surmised that both EPA and DHA increase HDL<sub>2</sub> choles-

terol because both fatty acids increased the ratio of HDL cholesterol to apo A-I. DHA increased HDL cholesterol and EPA decreased apo A-I, suggesting an increased surface-to-core ratio of the HDL particle and a redistribution of the HDL subclasses toward the larger HDL<sub>2</sub> particles (41). The mechanisms by which DHA increases HDL cholesterol are not known, but may be related to alterations in lipid transfer protein activity, which decreases after n-3 fatty acid supplementation (41). In epidemiologic terms, the increase in HDL<sub>2</sub> cholesterol could have a marked effect on the incidence of cardiovascular disease, given that HDL<sub>2</sub> cholesterol may be the subfraction of HDL cholesterol that may be most protective against coronary heart disease (42).

Although the LDL-cholesterol concentration increased after EPA and DHA intakes, the increase was significant only after DHA. The increased LDL-cholesterol concentration may relate to the hypotriglyceridemic effects of these fatty acids (43). n-3 Fats reduce hepatic VLDL synthesis, VLDL secretion, or both with the result that the smaller VLDL particles formed are more readily converted to LDL than are the larger VLDL particles (44). Smaller VLDL particles can also compete with LDL for uptake by LDL receptors. A down-regulation of the LDL receptor has been reported in some but not all studies (43).

LDL particle size increased significantly with DHA supplementation, a result that might be expected to contribute to a reduction in atherogenic risk. Ours is the first report showing a specific effect of DHA on LDL particle size, although others have shown increased LDL particle size after n-3 fatty acid supplementation (45, 46). Small, dense LDL particles are associated with an increased risk of coronary artery disease (47) and an increase in plasma triacylglycerol concentrations (48). Both

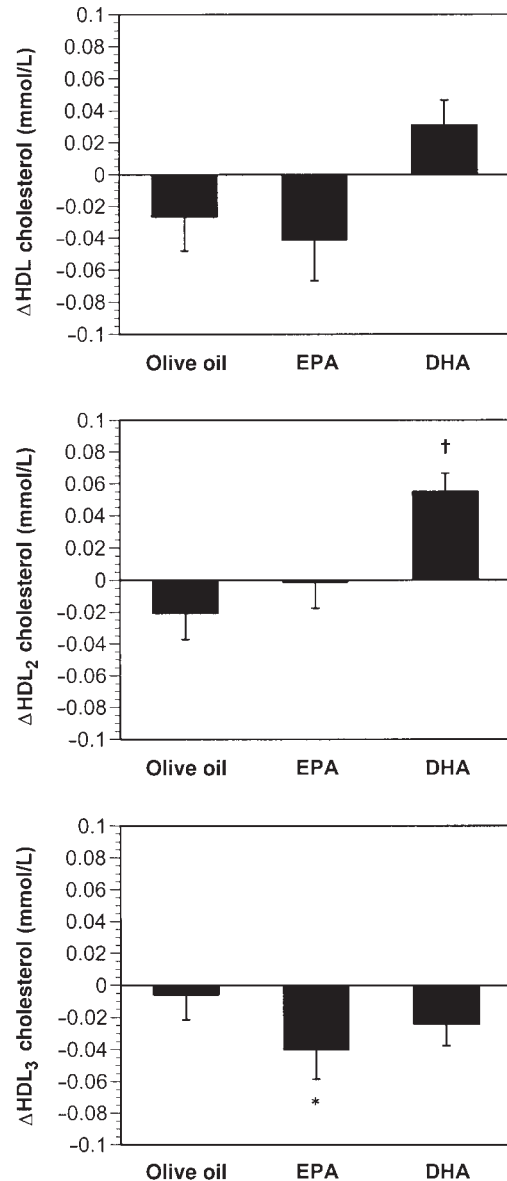


**FIGURE 3.** Mean ( $\pm$ SEM) changes in serum total cholesterol, LDL cholesterol, and triacylglycerols from baseline to the end of the intervention in the olive oil (control;  $n = 20$ ), eicosapentaenoic acid (EPA;  $n = 19$ ), and docosahexaenoic acid (DHA;  $n = 17$ ) groups. A general linear model was used to assess treatment effects on postintervention values adjusted for baseline values. \* $\dagger$ Significantly different from the olive oil group: \* $P < 0.05$ ,  $\dagger P < 0.01$ .

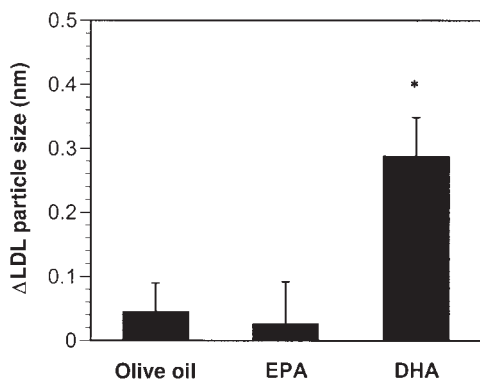
triacylglycerols and HDL cholesterol are major determinants of LDL particle size (49), partly because the exchange of triacylglycerols from VLDL for cholesterol ester in LDL, which is mediated by cholesteryl ester transfer protein (CETP). It is possible that as serum triacylglycerols decrease after  $n-3$  fatty acid supplementation, fewer triacylglycerols are transferred to LDL by CETP, reducing the formation of triacylglycerol-enriched LDL, which minimizes the opportunity for lipoprotein lipase to convert large LDL particles to small LDL particles. This hypothesis is supported by reports of reduced CETP activity after  $n-3$  fatty acid supplementation (41). Given the similarity in triacyl-

glycerol lowering by EPA and DHA, our results may be related to a more pronounced effect of DHA on CETP activity.

Olive oil supplementation did not alter plasma or platelet phospholipid oleic acid and other fatty acid concentrations; therefore, its use as a placebo was justified (5). Supplementation with EPA increased plasma and platelet phospholipid EPA and DPA concentrations, whereas the concentration of DHA was decreased. These findings can be explained by the inhibitory effect of EPA on  $\Delta^6$ -desaturase, which converts DPA to DHA (50), and confirm results of previous studies that humans do not synthesize DHA from EPA unless, perhaps, the EPA concentration is high (19).



**FIGURE 4.** Mean ( $\pm$ SEM) changes in serum HDL, HDL<sub>2</sub>, and HDL<sub>3</sub> cholesterol from baseline to the end of the intervention in the olive oil (control;  $n = 20$ ), eicosapentaenoic acid (EPA;  $n = 19$ ), and docosahexaenoic acid (DHA;  $n = 17$ ) groups. A general linear model was used to assess treatment effects on postintervention values adjusted for baseline values. \* $\dagger$ Significantly different from the olive oil group: \* $P < 0.05$ ,  $\dagger P < 0.01$ .



**FIGURE 5.** Mean ( $\pm$ SEM) changes in LDL particle size from baseline to the end of the intervention in the olive oil (control;  $n = 20$ ), eicosapentaenoic acid (EPA;  $n = 19$ ), and docosahexaenoic acid (DHA;  $n = 17$ ) groups. ANOVA was used to assess treatment effects on postintervention values adjusted for baseline values. \*Significantly different from the olive oil group,  $P < 0.01$ .

The accumulation of DPA may represent a temporary storage site for surplus EPA. DHA supplementation increased plasma and platelet DHA, and to a lesser extent EPA, in phospholipids, suggesting retroconversion of DHA to EPA. These findings agree with those of previous reports (17, 19) that suggest that  $\approx 9\%$  of dietary DHA is retroconverted to EPA (51). Interestingly, DPA decreased with DHA supplementation, suggesting that the increase in EPA is not caused solely by retroconversion, but also by decreased elongation of EPA. The reduced  $n-6$  and  $n-9$  fatty acids observed in the present study and in other studies (17–19) support an inhibitory role of EPA and DHA on  $\Delta^5$ -,  $\Delta^6$ -, and  $\Delta^9$ -desaturase enzymes (52), respectively. These findings also indicate selectivity of EPA and DHA incorporation into plasma and cellular membrane lipid stores (17).


The benefits of  $n-3$  fatty acids have to be weighed against the potential for impaired glucose tolerance, particularly in patients with type 2 diabetes (20–22), although no adverse effect has been seen in healthy volunteers or in hypertensive (25) or dyslipidemic patients (24). We showed in patients with type 2 diabetes that, under carefully controlled dietary conditions,  $n-3$  fatty acids can lead to a deterioration in glycemic control (23). This effect, however, was prevented by a moderate exercise program.

Both EPA and DHA supplementation increased fasting insulin, but only EPA increased fasting glucose. These results are consistent with a differential effect of EPA and DHA on glucose responses in humans. In contrast, lower doses of EPA (900 and 1800 mg/d) did not change fasting plasma glucose or glycated hemoglobin concentrations in patients with type 2 diabetes (53, 54).

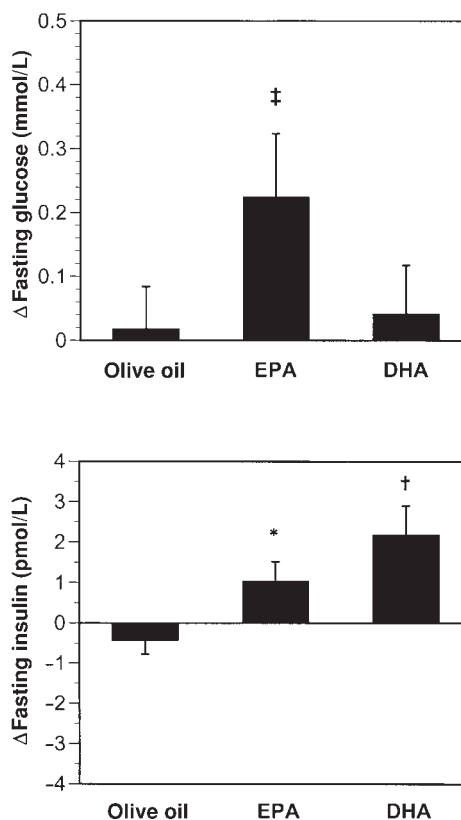
Mechanisms underlying the putative adverse effects of  $n-3$  fatty acids on glycemic control include an increase in hepatic glucose output, which may be related to an elevated flux of gluconeogenic precursors to the liver, increased plasma glucagon concentrations, changes in hepatic insulin or glucagon sensitivity, or decreased insulin secretion rates (20–22). The mechanisms responsible for the increase in fasting glucose after EPA but not after DHA supplementation are not known, but may be because EPA increases hepatic glucose production or decreases

hepatic insulin secretion more than does DHA. Further studies are required to resolve these issues.

In summary, this study showed differential effects of EPA and DHA on serum lipids, plasma and platelet fatty acids, and fasting glucose concentrations in mildly hypercholesterolemic but otherwise healthy men. We showed retroconversion of DHA to EPA, but not elongation of EPA to DHA. Both EPA and DHA decreased triacylglycerols and increased fasting insulin. Only DHA increased HDL cholesterol, particularly the HDL<sub>2</sub> subfraction. Despite an increase in LDL cholesterol after DHA supplementation, LDL particle size increased—a finding that may be favorable. Furthermore, EPA but not DHA increased fasting glucose concentrations.

These findings may help clarify which of the  $n-3$  fatty acids is responsible for the protective mechanisms of dietary  $n-3$  fats on cardiovascular disease. They suggest that, despite an increase in LDL cholesterol after DHA supplementation, the increased LDL particle size may represent a shift to less atherogenic particles, in which case the parallel increase in HDL<sub>2</sub> cholesterol and decrease in triacylglycerol may represent a more favorable lipid profile than that seen after EPA supplementation. 

We acknowledge Danny Bao, Lynette McCahon, and Ken Robertson for technical assistance; Jessie Prestage for nursing assistance; Nardia Ward for dietary counseling; and George Dragicevic for technical assistance with LDL particle sizing.



**FIGURE 6.** Mean ( $\pm$ SEM) changes in fasting serum glucose and insulin from baseline to the end of the intervention in the olive oil (control;  $n = 20$ ), eicosapentaenoic acid (EPA;  $n = 19$ ), and docosahexaenoic acid (DHA;  $n = 17$ ) groups. A general linear model was used to assess treatment effects on postintervention values adjusted for baseline values. \*<sup>†</sup>Significantly different from the olive oil group: \* $P < 0.05$ , <sup>†</sup> $P < 0.01$ , <sup>‡</sup> $P = 0.062$ .



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