

# Effects of purified eicosapentaenoic and docosahexaenoic acids on glycemic control, blood pressure, and serum lipids in type 2 diabetic patients with treated hypertension<sup>1-3</sup>

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## ABSTRACT

**Background:** n-3 Fatty acids lower blood pressure, improve lipids, and benefit other cardiovascular disease risk factors. Effects on glycemia in patients with type 2 diabetes are uncertain.

**Objective:** We determined whether purified eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have differential effects on glycemic control, including insulin sensitivity and stimulated insulin secretion; 24-h ambulatory blood pressure; and serum lipids in type 2 diabetic patients with treated hypertension.

**Design:** In a double-blind, placebo-controlled trial of parallel design, 59 subjects were randomly assigned to consume 4 g EPA, DHA, or olive oil/d for 6 wk while continuing to consume their usual diet.

**Results:** Thirty-nine men and 12 postmenopausal women with a mean ( $\pm$  SE) age of  $61.2 \pm 1.2$  y completed the study. In comparison with the change from baseline in fasting glucose in the olive oil group, fasting glucose in the EPA and DHA groups increased  $1.40 \pm 0.29$  mmol/L ( $P = 0.002$ ) and  $0.98 \pm 0.29$  mmol/L ( $P = 0.002$ ), respectively. Neither EPA nor DHA had significant effects on glycated hemoglobin, fasting insulin or C-peptide, insulin sensitivity or secretion, or blood pressure. Serum triacylglycerols in the EPA and DHA groups decreased 19% ( $P = 0.022$ ) and 15% ( $P = 0.022$ ), respectively. There were no significant changes in serum total, LDL, or HDL cholesterol, although HDL<sub>2</sub> cholesterol in the EPA and DHA groups increased 16% ( $P = 0.026$ ) and 12% ( $P = 0.05$ ), respectively. HDL<sub>3</sub> cholesterol decreased 11% ( $P = 0.026$ ) with EPA supplementation.

**Conclusions:** EPA and DHA had similar benefits on lipids but adverse effects on short-term glycemic control in hypertensive diabetic patients. The overall implications for cardiovascular disease require long-term evaluation. *Am J Clin Nutr* 2002;76:1007-15.

**KEY WORDS** Eicosapentaenoic acid, docosahexaenoic acid, type 2 diabetes, fish oil, glucose, blood pressure, lipids, n-3 fatty acids

## INTRODUCTION

There is considerable evidence for a protective effect of dietary n-3 fatty acids in the prevention of heart disease (1), especially in high-risk populations (2). Fish oils improve dyslipidemia (3) and vascular (4) and platelet function (5) and lower blood pressure in hypertensive patients (6, 7). In addition, insulin sensitivity has been positively correlated with concentrations of n-3 fatty acids in skeletal muscle (8). In 2 trials in patients with coronary

artery disease (9, 10), n-3 fatty acids from fish or fish oil decreased the risk of sudden cardiac death, an effect that may be related to a reduction in heart rate and in proneness to ventricular arrhythmia (11). Patients with type 2 diabetes have an increased risk of cardiovascular disease (12), and hypertension, hyperlipidemia, and insulin resistance are independent risk factors for macrovascular disease in type 2 diabetes (12-14). Therefore, an increased intake of n-3 fatty acids may be of particular benefit to this population.

Early reports from several uncontrolled studies, however, raised the possibility of an adverse effect of high doses of fish oils on glycemic control in type 2 diabetes (15-17). This was not confirmed in larger controlled studies in which subjects consumed 1.8-6.0 g n-3 fatty acids/d (18, 19). The importance of this relates to evidence that the level of glycemia in type 2 diabetic patients predicts all-cause and cardiovascular disease mortality (20) and that intensive blood glucose control decreases the risk of microvascular complications (21). Consequently, some experts urge caution in the use of n-3 fatty acids in type 2 diabetic patients (22), whereas others encourage such use, albeit at doses  $< 3$  g/d (18, 19).

Eicosapentaenoic acid (EPA; 22:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) are the 2 principal n-3 fatty acids in marine oils. Recent data from human studies showed that these 2 fatty acids have differential effects on serum lipids and lipoproteins (23, 24), serum glucose (24), blood pressure (25), heart rate (25, 26), and endothelial function (27). In particular, we found that 4 g purified DHA/d, but not EPA, decreased blood pressure and heart rate in overweight hypercholesterolemic subjects (25), whereas EPA, but not DHA, increased fasting glucose concentrations (24).

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To date, no study has been conducted to examine the independent effects of supplementation with highly purified EPA or DHA on cardiovascular risk factors in type 2 diabetic patients. Given the continuing controversy regarding glycemic control, a comparison of pure EPA and DHA in type 2 diabetic patients with treated hypertension is desirable before such supplementation is considered for broader use in this population. The aim of this study was to determine the effects of purified EPA and DHA on glycemic control, blood pressure, heart rate, and lipid metabolism in type 2 diabetic patients with treated hypertension.

## SUBJECTS AND METHODS

### Study population

Men and postmenopausal women aged 40–75 y who were non-smokers, had type 2 diabetes mellitus (as defined by being treated with oral hypoglycemic medications or having a fasting glucose concentration  $> 7.0$  mmol/L or a nonfasting glucose concentration  $> 11.1$  mmol/L), and had received drug treatment for hypertension for  $\geq 3$  mo were recruited from the general community by media advertising. Entry criteria included glycated hemoglobin ( $Hb A_{1c}$ )  $< 9\%$ , systolic blood pressure (SBP)  $> 115$  and  $< 180$  mm Hg [measured on 2 separate days with the use of a Dinamap 1846 SX/P monitor (Critikon, Inc, Tampa, FL)], diastolic blood pressure (DBP)  $< 110$  mm Hg, not receiving insulin therapy, body mass index (in  $kg/m^2$ )  $< 35$ , and fasting serum cholesterol and triacylglycerols  $< 7.5$  mmol/L. All subjects also ordinarily consumed  $\leq 2$  fish meals/wk, did not take fish oil supplements, and drank  $< 40$  g ethanol/d. Subjects were excluded if they had a recent history (within 3 mo) of heart disease, angina, or major surgery; had a recent history (within 6 mo) of myocardial infarction or stroke; had significant liver or renal disease (plasma creatinine  $> 130 \mu mol/L$ ), macroproteinuria, or symptomatic autonomic neuropathy; smoked or had smoked within the past 2 y; or regularly used nonsteroidal antiinflammatory drugs. Patients who were taking lipid-lowering drugs, aspirin (on a regular basis), or antioxidant vitamins were not excluded but were allowed to continue taking the same dose. Fifty-nine of the 144 screened subjects satisfied the entry criteria. The Royal Perth Hospital Ethics Committee approved the study, and all subjects gave written consent.

### Study design and interventions

Baseline measurements were collected during a 3-wk period, during which all subjects maintained their usual diet. They were then randomly assigned to one of 3 groups after stratification by sex, age, and body mass index. Capsules containing 4 g EPA, DHA, or olive oil placebo were given once a day with the evening meal for 6 wk. Subjects and investigators were blinded to the treatment. The capsules, which contained purified EPA ethyl ester ( $\approx 96\%$ ), DHA ethyl ester ( $\approx 92\%$ ), or olive oil ( $\approx 75\%$  oleic acid ethyl ester), were provided by the Fish Oil Test Materials Program and the US National Institutes of Health Department of Commerce. There was only a negligible amount of EPA ethyl ester within the DHA capsules ( $\approx 0.5\%$ ).

### Nutritional methods and lifestyle assessment

Subjects were instructed not to change their usual diets, alcohol intake, level of physical activity, or other lifestyle factors throughout the intervention period. Before the baseline period, a dietitian gave written and verbal instructions to the subjects on

how to keep accurate dietary records, including how to weigh or measure foods. A 3-d dietary record (2 weekdays and 1 weekend day) and a lifestyle questionnaire including history of illness, alcohol intake, medications, and physical activity were completed at baseline and after the intervention period. Dietary records were analyzed by using DIET/1 (version 4; Xyris, Brisbane, Australia), which is based on the Food Composition Database NUTTAB 1995A (28). Weight, alcohol intake, changes in physical activity and medication, and any illness were recorded each week during baseline and at weeks 2, 4, 5, and 6 of the intervention.

### Biochemical measurements

After the subjects fasted overnight, venous blood samples for measurement of serum glucose, insulin, C-peptide, lipids, and lipoproteins were taken twice (separated by 7 d): at baseline and at the end of the intervention. All samples were collected while the subjects rested in a supine position for 10 min. Serum glucose was measured within 12 h of collection with an automated Technicon Axon Analyzer (Bayer Diagnostics, Sydney, Australia) by using a hexokinase method. Serum insulin was measured by radioimmunoassay with an automated immunoassay analyzer (Tosoh Corporation, Tokyo). C-peptide was measured with the use of a solid-phase chemiluminescent assay (Diagnostic Products Corporation, Los Angeles).  $Hb A_{1c}$  was measured before and after intervention with the use of HPLC (Bio-Rad Laboratories, Sydney, Australia). The assay precision for glucose and insulin were described previously (24). The CVs for serum C-peptide at 0.56 and 2.75 nmol/L were 5.9% and 5.3%, respectively. The between-batch precision for  $Hb A_{1c}$  was  $< 1.0\%$ . Serum triacylglycerols, total cholesterol, total HDL cholesterol, and HDL<sub>2</sub>- and HDL<sub>3</sub>-cholesterol subfractions were measured as previously described (24). Briefly, serum concentrations of total cholesterol and triacylglycerols were determined enzymatically, HDL-cholesterol concentrations were determined on a heparin-manganese supernatant, and HDL<sub>2</sub>- and HDL<sub>3</sub>-cholesterol concentrations were determined by using a single-precipitation procedure. LDL-cholesterol concentrations were calculated by using the Friedewald formula (29), which is valid for triacylglycerol concentrations  $< 3.5$  mmol/L. Serum for insulin, lipid, and lipoprotein analyses was snap-frozen in liquid nitrogen and stored at  $-80^\circ C$ . Samples from baseline and at the end of the intervention were measured in a single assay to minimize interassay variation.

### Plasma phospholipid fatty acids

Total  $n-3$  fatty acids measured in plasma phospholipids included docosapentaenoic acid (DPA; 20:5 $n-3$ ), EPA, and DHA, and  $n-6$  fatty acids included linoleic acid (18:2 $n-6$ ), dihomo  $\gamma$ -linolenic acid (20:3 $n-6$ ), arachidonic acid (20:4 $n-6$ ), and adrenic acid (22:4 $n-6$ ) (30). Briefly, the phospholipid fraction was obtained from total lipid extracts by thin layer chromatography. Fatty acid methyl esters were prepared by treating phospholipid extracts with 4% (by vol)  $H_2SO_4$  in methanol at  $90^\circ C$  for 20 min and were analyzed by gas-liquid chromatography with a model 5980A gas chromatograph equipped with a 3393A computing integrator (Hewlett-Packard, Rockville, MD). Peaks were identified by comparing them with those of a known standard mixture. Individual fatty acids were calculated as a relative percentage, with the evaluated fatty acids set at 100%.

### Insulin sensitivity and insulin secretion

Insulin sensitivity and insulin secretion were assessed with the use of the low-dose insulin and glucose infusion test (31). This is

**TABLE 1**  
Characteristics of the diabetic patients at baseline<sup>1</sup>

Characteristic	Olive oil (n = 12 M, 4 F)	EPA (n = 14 M, 3 F)	DHA (n = 13 M, 5 F)
Age (y)	61.5 ± 7.6	61.2 ± 9.6	60.9 ± 8.2
Duration of diagnosed diabetes (y)	6.9 ± 7.5	4.2 ± 0.9	4.5 ± 3.4
BMI (kg/m <sup>2</sup> )	29.9 ± 4.0	27.9 ± 3.4	30.6 ± 3.1
Waist-to-hip ratio	0.96 ± 0.11	0.92 ± 0.06	0.92 ± 0.05
Glucose (mmol/L)	8.0 ± 1.6	7.5 ± 1.8	8.2 ± 1.0
Hb A <sub>1c</sub> (%)	7.1 ± 1.6	7.1 ± 1.0	7.5 ± 0.7
Blood pressure (mm Hg) <sup>2</sup>			
Systolic	128 ± 16	133 ± 14	133 ± 13
Diastolic	70 ± 8	73 ± 8	71 ± 8
Serum lipids (mmol/L)			
Cholesterol	4.6 ± 0.7	4.5 ± 1.0	4.5 ± 0.7
LDL cholesterol	2.7 ± 0.5	2.7 ± 0.8	2.7 ± 0.5
HDL cholesterol	1.06 ± 0.22	1.21 ± 0.35	0.99 ± 0.20
Triacylglycerols	1.7 ± 0.6	1.3 ± 0.7	1.6 ± 0.6

<sup>1</sup> $\bar{x} \pm \text{SD}$ . EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; Hb A<sub>1c</sub>, glycated hemoglobin. There were no significant differences between the groups (ANOVA).

<sup>2</sup> Average of 10 readings made on 2 separate days in the clinic with the use of a Dinamap 1846 SX/P monitor (Critikon, Inc, Tampa, FL) while the subjects were supine.

a modified version of the insulin and glucose infusion test developed by Heine et al (32); this modified version uses slightly lower doses of both insulin and glucose (25 compared with 50 mU · kg<sup>-1</sup> · h<sup>-1</sup> and 4 compared with 6 mg · kg<sup>-1</sup> · min<sup>-1</sup>). Measures of insulin sensitivity and insulin secretion obtained via this method correlate well with those obtained with the euglycemic and hyperglycemic clamp ( $r = 0.90$ ,  $P < 0.0001$  and  $r = 0.82$ ,  $P < 0.001$ , respectively) (31). A fasting blood sample was taken immediately before the test for glucose, insulin, and C-peptide, and additional blood samples were collected during the test at 5, 10, 15, 60, 120, 125, 130, 135, 140, 145, and 150 min. Samples were collected in 2-mL evacuated tubes; after clotting, the samples were centrifuged at 3500 rpm in a Universal 30 RF (Hettich, Tuttlingen, Germany) for 15 min at room temperature, and the serum was stored at -80°C for analysis of insulin, glucose, and C-peptide.

Insulin sensitivity during the low-dose insulin and glucose infusion test was calculated using the formula

$$\text{Insulin sensitivity index} = \text{glucose}_{\text{inf}} / (\text{glucose}_{\text{ss}} \times \text{insulin}_{\text{ss}})$$

where glucose<sub>inf</sub> is the glucose infusion rate, glucose<sub>ss</sub> is the steady state glucose concentration during the last 30 min of the test, and insulin<sub>ss</sub> is the steady state insulin concentration during the same period. Insulin secretion was evaluated by using the incremental area under the serum C-peptide concentration curve during the first 15 min.

### Self-monitoring of blood glucose

A nurse experienced in diabetes management trained the subjects to perform self-monitoring of blood glucose by using their own portable home monitor or one supplied through the study (Advantage, Castle Hill, Australia). Monitors were calibrated and checked for accuracy against a monitor used in the diabetic clinic of the Royal Perth Hospital. The timing of glucose measurements (time-of-glucose measurement) was as follows: after an overnight fast, 2 h before lunch, 2 h after lunch, and 2 h after the evening

meal on 4 d of each week during the baseline period and during the 6-wk intervention. The area under the curve was used to assess differences between the baseline (the mean of 3 wk; reported as week 0) and postintervention (the mean of weeks 1–6) values.

### Ambulatory blood pressure monitoring

Ambulatory blood pressure was monitored over 24 h at baseline and at the end of the intervention with an ambulatory blood pressure monitoring system (Accutrack II, model 104; Suntech Medical Instruments, Raleigh, NC). The recorder was preset to record SBP and DBP and heart rate every 30 min during waking hours (daytime) and hourly during sleep (nighttime). SBP and DBP records were not visible to the subjects. Recordings associated with a test code and those with a difference of <20 mm Hg between SBP and DBP were excluded from analysis. Subjects with <70% valid readings over the 24-h scan were excluded from analysis.

### Statistical analysis

Data were analyzed by using SPSS (SPSS Inc, Chicago) with general linear models to assess the effects of EPA or DHA relative to those of olive oil after adjustment for baseline values. Self-monitored blood glucose was assessed by using general linear models of the area under the curve. Curves for each group were constructed of the mean value of the 4 daily glucose measurements for each subject for each week. Week 0 was taken as the mean of the 3 wk of the baseline period, and the area-under-the-curve analysis used all mean weekly values from weeks 1 to 6. All variables were tested for normality by using histograms and Kolmogorov-Smirnov statistics. There were no nonnormal data either before or after the intervention. Significance levels were adjusted for multiple comparisons by using the Bonferroni method. Differences between the 3 groups were considered significant when  $P < 0.05$  (after Bonferroni adjustment). All significance levels are reported after adjustment for multiple comparisons. All values are reported as means ± SEMs except for the characteristics of the patients at baseline, which are reported as means ± SDs.

## RESULTS

### Study population

Fifty-one of the 59 randomly assigned subjects completed the study. Five subjects were withdrawn because their family practitioner made changes in their antihypertensive or diabetic medication, 2 subjects were withdrawn because of time commitments, and one was withdrawn because of an illness unrelated to the protocol. The characteristics of the patients confirmed that the groups were well matched for all entry criteria (Table 1), and there were no significant differences between the groups in either the type or number of lipid-lowering, antihypertensive, or oral hypoglycemic medications. The antihypertensive medications taken by the subjects were angiotensin-converting enzyme inhibitors (55%), calcium channel blockers (39%), angiotensin II receptor antagonists (24%), β-blockers (18%), diuretics (14%), and α-blockers (6%). One, 2, or ≥3 antihypertensive agents were used by 27%, 51%, and 24% of the subjects, respectively. The subjects also took oral hypoglycemic agents in the form of biguanides (53%), sulfonylureas (49%), and α-2 glucosidase inhibitors (2%), and 1, 2, or 3 oral hypoglycemic agents were used by 22%, 55%, and 24% of the subjects, respectively. Statins and fibrates were taken by 31% and 6% of the subjects, respectively, and 63% of the subjects used

**TABLE 2**Measures of glycemic control, insulin production, and insulin sensitivity at baseline and after the intervention<sup>1</sup>

	Olive oil (n = 16)	EPA (n = 17)	DHA (n = 18)	Treatment effect <sup>2</sup>	
				EPA	DHA
Fasting glucose (mmol/L)					
Baseline	7.96 ± 0.40	7.46 ± 0.44	8.25 ± 0.23		
After intervention	7.55 ± 0.34	8.49 ± 0.53	8.80 ± 0.29	1.40 ± 0.29 <sup>3</sup>	0.98 ± 0.29 <sup>3</sup>
Fasting insulin (mU/L)					
Baseline	14.57 ± 1.94	14.16 ± 1.76	16.54 ± 2.11		
After intervention	13.69 ± 1.92	13.84 ± 1.72	15.98 ± 1.67	−0.47 ± 1.29	−0.75 ± 1.28
Fasting C-peptide (nmol/L)					
Baseline	1.14 ± 0.11	0.95 ± 0.10	1.19 ± 0.13		
After intervention	0.99 ± 0.06	0.93 ± 0.09	1.11 ± 0.09	0.05 ± 0.08	0.09 ± 0.08
Hb A <sub>1c</sub> (%)					
Baseline	7.14 ± 0.15	7.14 ± 0.25	7.48 ± 0.17		
After intervention	7.04 ± 0.15	7.21 ± 0.26	7.33 ± 0.20	0.18 ± 0.14	0.03 ± 0.15
Insulin sensitivity index <sup>4</sup>					
Baseline	4.70 ± 0.53 [13]	7.22 ± 1.72 [14]	4.46 ± 0.51 [15]		
After intervention	4.39 ± 0.43 [13]	6.41 ± 1.32 [14]	4.89 ± 0.64 [15]	0.50 ± 0.91	0.65 ± 0.87
Insulin secretion <sup>5</sup>					
Baseline	−0.28 ± 0.43 [13]	0.32 ± 0.61 [14]	0.65 ± 0.73 [15]		
After intervention	1.08 ± 0.94 [13]	0.63 ± 0.49 [14]	0.93 ± 0.54 [15]	−0.54 ± 0.94	−0.32 ± 0.93

<sup>1</sup> $\bar{x} \pm \text{SEM}$ . There were no significant differences between the groups in baseline measures as assessed by ANOVA. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; Hb A<sub>1c</sub>, glycated hemoglobin.

<sup>2</sup>Changes in each of the 2 treatment groups compared with those in the olive oil group after adjustment for baseline values.

<sup>3</sup> $P = 0.002$ .

<sup>4</sup>Determined with the use of the low-dose insulin and glucose infusion test. Normal range: 22.6–50.2 [(mL · kg<sup>−1</sup> · min<sup>−1</sup>) × 10<sup>−3</sup>]/(pmol/L). *n* in brackets.

<sup>5</sup>Change in the area under the curve for serum C-peptide concentrations during the first 15 min of the low-dose insulin and glucose infusion test. Normal range: 0.58–5.7 nmol · min/L. *n* in brackets.

neither of these medications. Twenty-nine percent of the population received regular aspirin therapy.

### Diet and lifestyle

There were no significant differences between the groups in total energy intake, macronutrient intake, and body weight at baseline, and no significant changes took place during the intervention (data not shown). Medication doses, alcohol intake, and physical activity remained unchanged in each group.

### Plasma phospholipid fatty acids

Phospholipid fatty acid composition is expressed as a percentage of the total fatty acids. At baseline, EPA accounted for 1.9 ± 0.1%, 1.6 ± 0.2%, and 1.7 ± 0.2% of plasma phospholipid fatty acids in the olive oil, EPA, and DHA groups, respectively. DHA was present as 4.9 ± 0.2%, 4.3 ± 0.3%, and 4.3 ± 0.4% of fatty acids and DPA as 3.2 ± 0.1%, 3.1 ± 0.1%, and 3.1 ± 0.2% in the olive oil, EPA, and DHA groups, respectively. There were no significant changes in fatty acid composition in the control group. EPA supplementation increased EPA and DPA by 540% ( $P < 0.01$ ) and 69% ( $P < 0.01$ ), respectively, without significantly changing DHA (7% decrease). In the DHA group, DHA and EPA increased by 156% ( $P < 0.01$ ) and 64% ( $P < 0.01$ ), respectively, whereas DPA decreased by 17% ( $P < 0.01$ ).

### Serum glucose, glycated hemoglobin, fasting insulin, and C-peptide

Fasting glucose concentrations at the end of the intervention were significantly higher than at baseline in both the EPA and DHA groups ( $P < 0.001$  and  $P < 0.005$ , respectively). In comparison with the change in the olive oil group, fasting glucose

increased 1.40 mmol/L ( $P = 0.002$ ) and 0.98 mmol/L ( $P = 0.002$ ) in the EPA and DHA groups, respectively, after adjustment for baseline values (Table 2). The increases remained significant after further adjustment for age, sex, and body mass index. Relative to olive oil, neither EPA nor DHA had any significant effects on fasting insulin, C-peptide, or Hb A<sub>1c</sub> (Table 2).

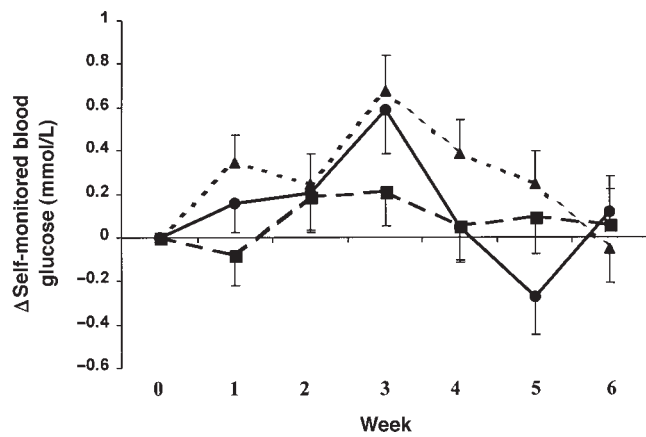
### Self-monitoring of blood glucose

Self-monitored blood glucose at baseline was significantly higher in the DHA group than in the olive oil group (8.6 ± 0.2 mmol/L compared with 7.8 ± 0.2 mmol/L,  $P = 0.01$  after adjustment for sex and time-of-glucose measurement). After adjustment for baseline values, sex, and time-of-glucose measurement, the mean glucose area under the curve showed a trend toward higher values after EPA supplementation ( $P = 0.09$ ) than after supplementation with olive oil, but there was no significant change after DHA supplementation. As shown in Figure 1, the average increases in the EPA and DHA groups peaked at week 3 but returned to baseline values by week 6.

### Insulin sensitivity and stimulated insulin secretion

The mean CV for glucose<sub>ss</sub> in the 3 groups was 3.64 ± 0.56% at baseline and 3.54 ± 0.70% after the intervention. The mean CV for insulin<sub>ss</sub> in the 3 groups was 11.61 ± 1.46% at baseline and 11.04 ± 1.56% after the intervention. For each of the groups, the values for glucose<sub>ss</sub>, insulin<sub>ss</sub>, insulin sensitivity index, and stimulated insulin secretion after the intervention did not differ significantly from those at baseline. In comparison with the changes in the olive oil group, there were no significant changes in insulin sensitivity index or insulin secretion during the intervention in the EPA or DHA groups (Table 2).





**FIGURE 1.** Mean ( $\pm$ SEM) change ( $\Delta$ ) in self-monitored blood glucose from baseline (week 0) throughout intervention (weeks 1–6) in the olive oil (■;  $n = 15$ ), eicosapentaenoic acid (EPA; ▲;  $n = 17$ ), and docosahexaenoic acid (DHA; ●;  $n = 18$ ) groups. A general linear model using area under the curve (from week 0 to week 6) was used to assess treatment effects. After adjustment for baseline values, sex, and time of glucose measurement,  $P = 0.09$  for differences between the EPA and olive oil groups and  $P = 0.47$  for differences between the DHA and olive oil groups.

#### Ambulatory blood pressure and heart rate

The mean values for 24-h SBP, DBP, and heart rate in each group at baseline and after the intervention are shown in **Table 3**. One subject in the DHA group was not tested after the intervention because he suffered a mild stroke just before the end of the study. Three subjects (one in each group) were removed from the final analysis because of an insufficient number of valid readings ( $<70\%$ ) over the 24-h period either at baseline or after the intervention. There were no significant differences in SBP, DBP, or heart rate between the groups at baseline. In comparison with the changes in the olive oil group, there were no significant changes in 24-h SBP or DBP in either the EPA or DHA groups. There were also no significant differences between the groups in SBP or DBP during waking hours or during sleep (data not shown). There were nonsignificant decreases in 24-h heart rate during waking hours

and during sleep in both the EPA and DHA groups compared with the changes in the olive oil group (data not shown).

#### Serum lipids and lipoproteins

The mean values for serum lipids and lipoproteins in each group at baseline and after the intervention are shown in **Table 4**. Fasting triacylglycerols at baseline were  $<3.5$  mmol/L in each of the groups, implying reliable assessment of LDL cholesterol with the use of the Friedwald formula (29). At baseline, HDL<sub>2</sub>-cholesterol concentrations were significantly lower in the DHA group than in the EPA group ( $P < 0.05$ ). There were no significant differences between the groups at baseline in any of the other variables. After adjustment for baseline values, fasting triacylglycerol concentrations decreased 19% with EPA supplementation ( $P = 0.022$ ) and 15% with DHA supplementation ( $P = 0.022$ ; Table 4) in comparison with the change in the olive oil group. Neither EPA nor DHA had an effect on serum total cholesterol or LDL-cholesterol concentrations or on the ratio of LDL to HDL cholesterol. While there were no significant changes in total HDL-cholesterol concentrations, in comparison with the changes in the olive oil group, HDL<sub>2</sub>-cholesterol concentrations in the EPA and DHA groups increased 16% ( $P = 0.026$ ) and 22% ( $P = 0.05$ ), respectively, and HDL<sub>3</sub>-cholesterol concentrations decreased 11% ( $P = 0.026$ ) and 6% ( $P = 0.33$ ), respectively.

#### DISCUSSION

This study showed that in patients with type 2 diabetes and treated hypertension, 4 g purified EPA or DHA/d had significant adverse effects on glycemic control as measured by fasting glucose. There was also a trend toward increased self-monitored blood glucose with EPA supplementation, although the effects appeared to be transient. Neither EPA nor DHA had significant effects on fasting insulin or C-peptide, insulin sensitivity, or stimulated insulin release. EPA and DHA affected serum lipids and lipoproteins similarly, with significant decreases in triacylglycerols and significant increases in HDL<sub>2</sub> cholesterol. EPA also significantly decreased HDL<sub>3</sub> cholesterol. There were no significant changes in SBP, DBP, or heart rate with either oil.

Our finding of mild impairment of glycemic control is supported by reports of a significant increase in fasting glucose after consumption of fish (33) or fish oils (34) in comparison with the

**TABLE 3**

Twenty-four-hour blood pressure and heart rate (HR) at baseline and after the intervention<sup>1</sup>

	Olive oil ( $n = 16$ )	EPA ( $n = 17$ )	DHA ( $n = 17$ )	Treatment effect <sup>2</sup>	
				EPA	DHA
24-h SBP (mm Hg)					
Baseline	135.9 $\pm$ 3.6	137.1 $\pm$ 4.1	138.5 $\pm$ 3.9		
After intervention	132.5 $\pm$ 2.8	133.7 $\pm$ 3.4	142.7 $\pm$ 4.8	1.3 $\pm$ 3.5	6.0 $\pm$ 3.5
24-h DBP (mm Hg)					
Baseline	73.0 $\pm$ 1.5	75.8 $\pm$ 2.2	71.8 $\pm$ 2.4		
After intervention	72.1 $\pm$ 1.3	74.6 $\pm$ 1.9	71.9 $\pm$ 1.8	0.6 $\pm$ 1.7	0.9 $\pm$ 1.7
24-h HR (beats/min)					
Baseline	72.3 $\pm$ 2.4	73.4 $\pm$ 3.4	71.4 $\pm$ 2.5		
After intervention	72.7 $\pm$ 2.3	69.8 $\pm$ 2.9	69.6 $\pm$ 2.6	-0.9 $\pm$ 2.6	-1.5 $\pm$ 2.6

<sup>1</sup> $\bar{x} \pm$  SEM. There were no significant differences between the groups in baseline measures as assessed by ANOVA. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SBP, systolic blood pressure; DBP, diastolic blood pressure.

<sup>2</sup>Changes in each of the 2 treatment groups compared with those in the olive oil group after adjustment for baseline values. There were no significant treatment effects.

**TABLE 4**Fasting serum lipids and lipoproteins at baseline and after the intervention<sup>1</sup>

	Olive oil (n = 16)	EPA (n = 17)	DHA (n = 18)	Treatment effect <sup>2</sup>	
				EPA	DHA
Cholesterol (mmol/L)					
Baseline	4.57 ± 0.17	4.48 ± 0.25	4.49 ± 0.15		
After intervention	4.61 ± 0.17	4.42 ± 0.21	4.55 ± 0.18	−0.12 ± 0.13	0.01 ± 0.13
Triacylglycerols (mmol/L)					
Baseline	1.74 ± 0.15	1.34 ± 0.18	1.62 ± 0.14		
After intervention	1.68 ± 0.14	1.11 ± 0.14	1.35 ± 0.13	−0.25 ± 0.09 <sup>3</sup>	−0.24 ± 0.09 <sup>3</sup>
LDL cholesterol (mmol/L)					
Baseline	2.71 ± 0.12	2.66 ± 0.20	2.74 ± 0.13		
After intervention	2.77 ± 0.11	2.68 ± 0.19	2.88 ± 0.14	−0.04 ± 0.10	0.08 ± 0.10
HDL cholesterol (mmol/L)					
Baseline	1.06 ± 0.06	1.21 ± 0.09	0.99 ± 0.05		
After intervention	1.07 ± 0.06	1.22 ± 0.09	1.03 ± 0.05	−0.01 ± 0.03	0.03 ± 0.03
HDL <sub>2</sub> cholesterol (mmol/L)					
Baseline	0.38 ± 0.04	0.49 ± 0.07	0.32 ± 0.03 <sup>4</sup>		
After intervention	0.38 ± 0.04	0.56 ± 0.08	0.38 ± 0.04	0.08 ± 0.03 <sup>3</sup>	0.07 ± 0.03 <sup>3</sup>
HDL <sub>3</sub> cholesterol (mmol/L)					
Baseline	0.68 ± 0.02	0.73 ± 0.03	0.67 ± 0.03		
After intervention	0.69 ± 0.03	0.66 ± 0.03	0.65 ± 0.03	−0.08 ± 0.03 <sup>3</sup>	−0.04 ± 0.03

<sup>1</sup> $\bar{x} \pm \text{SEM}$ . EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.<sup>2</sup>Changes in each of the 2 treatment groups compared with those in olive oil group (no adjustments were made for baseline values).<sup>3</sup> $P < 0.05$ .<sup>4</sup>Significantly different from the EPA group,  $P < 0.05$  (one-way ANOVA).

change after consumption of a placebo. Furthermore, we previously found a significant increase in both glycated hemoglobin and self-monitored blood glucose in overweight type 2 diabetic patients after a fish diet supplying 3.65 g n-3 fatty acids/d for 8 wk (35). We also recently reported a trend ( $P = 0.06$ ) toward increased fasting glucose in overweight, mildly hyperlipidemic men with 4 g purified EPA/d but not with DHA (24).

The disparate findings concerning effects on glycemic control in type 2 diabetic patients may be related to the dose of n-3 fatty acids (18), differences in oral diabetic medication, degrees of obesity and insulin resistance, the presence of other conditions such as hypertension that may also affect insulin sensitivity, and the lack of control of subjects' diets during intervention. Additionally, most controlled studies were of longer duration ( $\approx 12$  wk) than ours was, raising the possibility that the increase in blood glucose may be transient. In support of this, self-monitored blood glucose in both the EPA and DHA groups increased in the present study in the first 3 wk, before returning to baseline values thereafter. Previous studies showed a transient increase in fasting glucose (36) and glycated hemoglobin (15), although in the present study, fasting glucose remained elevated at 6 wk.

Despite the increases in fasting glucose, there were no differences in either insulin sensitivity or insulin secretion between groups after the intervention. This concurs with other controlled studies in either type 2 diabetic or nondiabetic subjects (37, 38). Only uncontrolled fish oil studies reported an improvement in insulin sensitivity and decreased stimulated insulin secretion (16, 39). Controlled studies showed no effect on fasting insulin, C-peptide, or stimulated insulin release in either diabetic or nondiabetic populations (34, 40). However, in hyperlipidemic subjects with mild hyperinsulinemia, 3.4 g fish oil/d for 6 mo caused a significant decrease in fasting insulin (41).

In view of the lack of significant changes in insulin sensitivity and secretion, an increase in hepatic glucose output seems to be

the most plausible explanation for the worsening of glycemic control in our study. Hepatic glucose production is highly correlated with the degree of fasting hyperglycemia in obese type 2 diabetic patients (42). No changes in hepatic insulin sensitivity have been reported with type 2 diabetic patients (37, 43), but glycemic control was also unaffected in those studies. However, in an uncontrolled study, hepatic glucose output increased in parallel with fasting glucose (16).

The triacylglycerol-lowering effect of n-3 fatty acids is well established (3) and may be related to an increase in hepatic glucose output. An increase in peroxisome proliferator-activated receptor  $\alpha$  with EPA supplementation leads to increased hepatic uptake and oxidation of free fatty acids (44), as well as increased fatty acid oxidation in skeletal muscle (45). The consequent decrease in free fatty acid availability would lead to decreased triacylglycerol synthesis, and an increase in hepatic free fatty acid oxidation could both increase hepatic gluconeogenesis (46) and decrease glucose oxidation via the Randle glucose-fatty acid cycle (47). Increases in hepatic mitochondrial  $\beta$ -oxidation occurred with EPA but not with DHA after both fatty acids elevated peroxisome proliferator-activated receptor  $\alpha$  in rat hepatocytes (44). This may explain the greater tendency of EPA to increase fasting glucose in our earlier study (24) and self-monitored blood glucose in the present study. However, caution should be used when extrapolating data from animal studies, and it remains to be determined whether n-3 fatty acids affect peroxisome proliferator-activated receptor  $\alpha$  in humans.

Neither EPA nor DHA decreased blood pressure in the present study. In contrast with our previous report in which DHA, but not EPA, significantly decreased 24-h ambulatory blood pressures in overweight, mildly hypercholesterolemic men (25), there was a trend toward increased blood pressure with DHA in the present study. Possible explanations for the lack of antihypertensive effect in our study were the concomitant use of other pharmacologic

agents, adequacy of glycemic control, increased blood pressure variability in these diabetic patients resulting in inadequate statistical power, and the choice of olive oil as placebo.

Most of the intervention studies showing the beneficial effect of fish oil on blood pressure included untreated hypertensive subjects (6). There was no effect on blood pressure, as measured with manual sphygmomanometry, with 2.7 g EPA/d and 1.8 g DHA/d given to nondiabetic patients with treated hypertension (48). However, we showed that a fish diet providing 3.65 g n-3 fatty acids/d decreases ambulatory blood pressures in treated hypertensive patients (7), suggesting that antihypertensive drug therapy per se should not obscure the response to n-3 fatty acids.

Increases in fasting glucose with both EPA and DHA may have contributed to the lack of hypotensive effect via an increase in reactive oxygen species, particularly superoxide, which is believed to play a role in the pathogenesis of hypertension (49). Reactive oxygen species may cause a decrease in available endothelial nitric oxide and endothelial dysfunction (50, 51), and hyperglycemia counteracts the antioxidant effects of glutathione in diabetic patients (52). Patients with type 2 diabetes have increased production of thromboxane (53), a potent vasoconstrictor that may be related to glycemic control (54).

Compared with a diet rich in n-6 fatty acids, a diet that includes olive oil improves blood pressure (55), arterial compliance (56), and fasting glucose (57) in diabetic patients. However, this was at doses substantially higher than the dose of placebo in the present study. Moreover, we have shown that when compared with olive oil, purified DHA significantly reduces blood pressure in nondiabetic subjects (25).

The recent GISSI Prevenzione trial (9) showed that even at low doses (1 g/d), n-3 fatty acids decrease the risk of sudden cardiac death by 45%, possibly by stabilizing myocardial membranes and reducing susceptibility to ventricular arrhythmias (11). This effect may be related to the reduction in heart rate usually found with fish and fish oils (7). The present study showed only a trend toward lower heart rates, which may have been related to the presence of unidentified autonomic neuropathy.

The decrease in triacylglycerols, without changes in total, LDL, or HDL cholesterol, is in accordance with most previous fish oil studies in type 2 diabetic patients (58). In nondiabetic subjects, purified EPA and DHA decreased triacylglycerols (23, 24) without significantly changing total cholesterol. In one report, DHA increased both HDL and LDL cholesterol, but the latter change was associated with an increase in LDL particle size, representing a shift to a less atherogenic particle (24). Although HDL<sub>2</sub> and HDL<sub>3</sub> cholesterol have usually remained unchanged in type 2 diabetic subjects after n-3 fatty acid supplementation (59), we observed a significant increase in HDL<sub>2</sub> cholesterol with both EPA and DHA. The antiatherogenic shift in HDL particle size may be the result of a decrease in the activity of cholesteryl ester transfer protein (60), which is known to be elevated in type 2 diabetes (61).

In summary, this study showed that over 6 wk both EPA and DHA led to a mild impairment of glycemic control in moderately obese type 2 diabetic patients with treated hypertension. The most likely explanation appears to be an increase in hepatic glucose output, although this was not directly assessed. Counterbalancing these potentially adverse effects were significant decreases in serum triacylglycerols and significant increases in HDL<sub>2</sub> cholesterol with both EPA and DHA.

These results suggest that 4 g purified EPA or DHA/d does not offer any substantial advantages over fish or mixed fish oil supplements

with respect to effects on glycemic control, lipids, or SBP and DBP in hypertensive patients with type 2 diabetes. The potentially beneficial effects on platelet function and inflammatory processes relevant to atherosclerosis mean that the overall benefits and risks of EPA and DHA will need to be assessed in longer term studies in which major morbidity and mortality are the primary outcome measures.



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