Effect of Dietary Fish and Exercise Training on Urinary F_2-Isoprostane Excretion in Non–Insulin-Dependent Diabetic Patients

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Despite the potential benefits of dietary treatment with marine ω3 fatty acids in cardiovascular disease, there remains concern with respect to their potential for increased lipid peroxidation. Thus far, data from in vivo studies are inconclusive. Increased lipid peroxidation has also been associated with acute exercise in some studies, but the methods have been nonspecific. The quantitation of F_2-isoprostanes provides a more reliable and useful assessment of in vivo lipid peroxidation. We therefore aimed to assess the independent and combined effects of dietary ω3 fatty acids and aerobic exercise training on urinary F_2-isoprostane levels in dyslipidemic non–insulin-dependent diabetic (NIDDM) patients. In a randomized controlled trial, 55 untrained, sedentary, dyslipidemic NIDDM patients were randomly assigned to a low-fat diet (30% of daily energy) with or without one daily fish meal (3.6 g ω3 fatty acids per day) and further randomized to either a moderate (55% to 65% maximal oxygen consumption [$\dot{V}O_2$max]) or light (heart rate < 100 bpm) exercise training program for 8 weeks. Twenty-four-hour urine samples from 49 subjects were collected for measurement of urinary F_2-isoprostanes by gas chromatography–mass spectrometry before and after intervention. The fish diets reduced urinary F_2-isoprostanes by 830 ± 321 pmol/24 h (20%, $P = .013$) relative to the low-fat diet alone. This effect was independent of age, gender, and body weight change. Moderate exercise training did not alter F_2-isoprostanes. These findings show that, at least in the short-term, exercise had no effect, whereas the inclusion of regular fish meals as part of a low-fat diet reduced in vivo lipid peroxidation in dyslipidemic NIDDM patients. This response could further complement the known benefits of ω3 fatty acids and exercise favoring a reduced cardiovascular risk in diabetic patients.

DIABETES MELLITUS is an independent risk factor for the development of atherosclerosis.[1] One hypothesis for the accelerated atherosclerosis of diabetes is that the modification of lipoproteins, particularly low-density lipoprotein (LDL), by oxidation, glycosylation, or both may induce endothelial injury and accelerate foam-cell formation by monocytes-macrophages in the arterial intima.[2] These processes are thought to be integral steps in the genesis of the atherosclerotic lesion. Incorporation of ω3 fatty acids into cellular membranes and lipoproteins has the potential to influence a variety of mechanisms underlying atherosclerosis such as lipid metabolism, endothelial function, vascular reactivity, monocyte-macrophage function, thrombosis, and inflammation.[3] Despite these benefits of ω3 fatty acids, concern remains with respect to the potential for increased lipid peroxidation,[4] based on the observation that the susceptibility of fatty acids to oxidation is proportional to their degree of unsaturation. However, the data, particularly in vivo, are inconclusive, in part due to limitations in the methodologies used.

Exercise plays an important role in the therapy of patients with non–insulin-dependent diabetes mellitus (NIDDM) through its known benefits on insulin sensitivity and a variety of cardiovascular disease risk factors.[5,6] Despite this, several studies, including a recent study in young men with type 1 diabetes, have demonstrated that free-radical production and subsequent lipid peroxidation increase even during acute moderate aerobic exercise, overwhelming the body's antioxidant defenses and resulting in oxidative stress.[7-9] This may be of particular concern for NIDDM patients, given their increased susceptibility to oxidative stress. In contrast, in nondiabetics, there is accumulating evidence that regular exercise training augments the body's antioxidant defenses and may decrease resting and exercise-induced oxidative stress as compared with the nontrained state.[10,11] It has also been suggested that physical fitness may have a protective effect against lipid peroxidation.[12,13] To our knowledge, the impact of regular aerobic exercise on oxidative stress in patients with NIDDM has not been investigated.

The F_2-isoprostanes consist of a series of chemically stable prostaglandin F_2 (PGF_2)-like compounds generated from the peroxidation of unsaturated fatty acids in membrane phospholipids independently of the cyclooxygenase enzyme.[14] The quantitation of F_2-isoprostanes is thought to provide a reliable and useful assessment of in vivo lipid peroxidation.[14] However, no study has examined the effect of ω3 fatty acids and/or exercise on F_2-isoprostane excretion. We now report a randomized controlled intervention trial examining the independent and combined effects of ω3 fatty acids derived from one daily fish meal and moderate-intensity aerobic exercise training on urinary F_2-isoprostane levels in dyslipidemic NIDDM patients consuming a reduced-fat (30% of total energy intake) diet.

SUBJECTS AND METHODS

Participants

One hundred twenty-seven nonsmoking subjects aged 30 to 65 years with treated (diet and/or medication) NIDDM were screened for the study. Of these, 55 (40 men and 15 women) met the following entry criteria: fasting serum triglycerides greater than 1.8 mmol/L and/or

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high-density lipoprotein cholesterol (HDL-C) less than 1.0 mmol/L; body mass index (BMI) less than 36 kg/m^2; no insulin therapy or lipid-lowering medication; no history or evidence of heart, liver, or renal disease, neuropathy, or retinopathy; nonasthmatic; and no orthopedic disorder that precludes exercise participation. On the basis of a life-style screening questionnaire, only subjects characterized as having a sedentary life-style (defined as <60 minutes of vigorous activity per week) for at least the previous 6 months were included. All subjects underwent a comprehensive medical examination including a medical history, physical examination, and resting 12-lead electrocardiogram. Antidiabetic and antihypertensive medications were continued during the study. The subjects' usual dietary habits, physical activity, alcohol consumption, and medication levels were encouraged throughout and were assessed by 24-hour diet records and questionnaires. The subjects provided written consent, and all methods and procedures were approved by the Human Rights Committee of the University of Western Australia.

Study Design and Interventions

The study design and diets are described in greater detail elsewhere.[15] Briefly, after an initial 4-week baseline period, all subjects consumed diets that were reduced in sodium intake to less than 100 mmol/d and supplied less than 30% of total energy as fat (<10% saturated fat). The study was a two-way factorial intervention of parallel design and 8 weeks' duration. Subjects were randomized to one of four treatment groups: a low-fat diet alone or including one fish meal daily, and within each of these treatment arms, either a moderate (55% to 65% of maximal oxygen consumption [$\dot{V}O_2$max]) or light (heart rate <100 bpm; control) exercise training group. The diets were individually tailored for each participant by a dietitian using the estimated energy intake obtained from two weighed 3-day food records performed during baseline. Participants who were randomized to eat fish included one daily fish meal as part of their low-fat diet. Diets and menus were designed to provide a variety of fish in order to maintain compliance. The subjects were required to eat approximately 100 g/d tuna, 50 g/d salmon, 100 g/d sardines, or 200 g/d Greenland turbot fillets, each of which provided approximately 3.6 g ω3 fatty acids per day. Weekly interviews with the dietitian and a weekly food checklist were used to assess compliance with the diets.

All subjects performed exercise training on 3 nonconsecutive days of the week in a supervised laboratory setting for 8 weeks. Participants assigned to moderate exercise performed stationary cycling on a bicycle ergometer for 30 minutes. A 5-minute warm-up and 5-minute cool-down (stationary cycling with no workload and stretching exercises) preceded and concluded each exercise session. The training intensity was individualized according to results achieved during maximum exercise testing. Initially (week 1), subjects exercised at an intensity corresponding to 50% to 55% $\dot{V}O_2$max, and thereafter at 55% to 65% $\dot{V}O_2$max (weeks 2 to 8). The light exercise protocol served as a control exercise program and consisted of stationary cycling with zero external resistance (unloaded cycling) for 10 minutes followed by a series of stretching/flexibility exercises for 30 minutes.

Other Measurements

At each laboratory visit and fortnightly throughout the intervention, the subjects were weighed without shoes using a calibrated beam-balance scale. The waist circumference at the level of the natural waist was determined before and after the intervention. Twenty-four-hour urine samples were collected at baseline and 48 hours after the final exercise period at completion of the intervention. Participants received written and verbal instructions on the correct procedures for sample collection. The methods for the laboratory-based assessment of platelet, plasma, and red blood cell phospholipid fatty acids, as well as glycemic control including fasting serum glucose, insulin, glycated hemoglobin, and glucose and insulin measured as the area under the oral glucose tolerance test curve, were previously reported.[15] All measurements were recorded at baseline and at the end of the intervention.

Analysis of Urinary F_2-Isoprostanes

The assay details have been recently reported[16] and are described in brief herein.

Purification. Urine (2 mL) and 8-iso-PGF$_{2\alpha}$-d$_4$ (5 ng, internal standard) were acidified to pH 3 with 2 mol/L HCl and applied to a preconditioned C_{18} Sep-Pak cartridge (Waters, Milford, MA), and the column was washed with water (pH 3, 10 mL), acetonitrile/water (15:85, 10 mL), and petroleum spirit (10 mL). The F_2-isoprostanes were eluted with ethyl acetate/petroleum spirit (50:50, 10 mL), and the eluate was dried over anhydrous $MgSO_4$ and then applied to a silica Sep-Pak cartridge. The column was washed with ethyl acetate (5 mL), and the F_2-isoprostane fraction that eluted with ethyl acetate/methanol (50:50, 5 mL) was evaporated to dryness, reconstituted in methanol (40 μL), and subjected to high-performance liquid chromatography (HPLC) on a LiChrospher 100 RP-18 125- × 4-mm column (Hewlett-Packard, Palo Alto, CA) using a Hewlett-Packard Series 1100 HPLC System connected to a Gilson FC-205 Fraction Collector (Gilson, Middleton, WI). The mobile phase was acetonitrile (solvent A) and 0.05% acetic acid in water (solvent B). Separation was performed at a flow rate of 1 mL/min and a linear gradient starting with 10% solvent A and ramping to 50% solvent A at 20 minutes and then 100% solvent A at 25 minutes and maintained for a further 5 minutes. The F_2-isoprostanes eluted between 14.8 and 15.8 minutes.

Derivatization. The F_2-isoprostane fraction was treated with pentafluorobenzylbromide (40 μL, 10% vol/vol in acetonitrile) and *N,N*-diisopropylethylamine (20 μL, 10% vol/vol in acetonitrile) at room temperature for 30 minutes. Excess reagents were removed under N_2, and the sample was then treated with *N,O*-bis-(trimethylsilyl)trifluoroacetamide + 1% trimethylchlorosilane ([BSTFA + TMCS] 99:1, 20 μL) and anhydrous pyridine (10 μL) at 45°C for 20 minutes. Excess reagents were evaporated under N_2.

Gas chromatography–mass spectrometry. Samples reconstituted in isooctane (25 μL) were analyzed on an HP 5890 Series II Plus gas chromatograph coupled to an HP 5989B mass spectrometer (Hewlett-Packard). Chromatography was performed with an HP 5MS column (30 m × 0.25 mm, 0.25 μm; Hewlett-Packard) with helium as the carrier gas. The negative ion chemical ionization used methane as the reagent gas at an ion source pressure of 1.8 mm Hg. The initial column temperature of 160°C was maintained for 1 minute, increased from 160°C to 300°C at 20°C/min, and maintained at 300°C for 17 minutes. The mass spectrometer monitored ions *m/z* 567, 569, and 573 for F_3-isoprostanes, F_2-isoprostanes, and 8-iso-PGF$_{2\alpha}$-d$_4$, respectively. The within- and between-assay reproducibility is 6.7% and 3.7%, respectively.

Statistical Analysis

The data were checked for frequency distribution using the SPSS software package (SPSS, Chicago, IL) and were logarithmically transformed when appropriate. One-way ANOVA was used to assess between-group comparisons at baseline. The main effects of fish consumption and moderate exercise from baseline to postintervention were assessed using the Proc General Linear Models ([GLM] SAS Institute, Cary, NC). All models were adjusted for the baseline measurement to avoid the effect of regression to the mean that leads to a change in a variable depending on its initial value.[17] To assess whether the effects were independent of age, gender, and change in body weight, other models included these variables as covariates. A probability level less than .05 was accepted as significant. Data are presented as the mean ± SEM or regression coefficient (B) and standard error (SEB) unless otherwise indicated.

RESULTS

Forty-nine of 55 subjects who commenced the study completed the 8-week intervention and were included in the analysis. There were 12 subjects in the control group, 12 in the group taking fish alone, 11 in the moderate exercise program alone, and 14 in the combined fish and moderate exercise group (Table 1). The groups were well matched for gender, age, BMI, duration of diabetes, and diabetic treatment regimen. There were no significant differences (ANOVA) between the groups for any of the baseline characteristics. Three subjects withdrew from the study during the baseline period due to changes in medication or an inability to successfully meet the time commitments. Another three subjects were excluded because of changes in diabetic medication, an inability to meet the dietary and time requirements of the study, or surgery that prevented the ascertainment of postintervention measurements. Medication levels were unchanged during the study in the remaining subjects, as assessed by fortnightly medication frequency questionnaires.

Diet, Weight, and Fitness Measures

Diet records and analyses of plasma, platelet, and red blood cell phospholipid fatty acids confirmed compliance with the diets, and questionnaires established that there were no significant changes in alcohol consumption or physical activity.[15] Detailed analyses of the total energy and macronutrient intake have been published previously.[15] All subjects completed at least 21 of 24 exercise sessions, with no major complications reported. Moderate exercise was associated with a reduction in the mean body weight of 1.3 ± 0.1 kg ($P < .05$) relative to the light exercise groups. The change in body weight by group was as follows: −0.6 ± 1.4 kg in the control group, −1.4 ± 1.7 kg in the fish group, −2.1 ± 1.4 kg in the moderate exercise group, and −2.4 ± 2.0 kg in the fish + moderate exercise group. There was a 12% improvement in aerobic fitness ($P < .001$) in the two moderate exercise groups compared with the light exercise groups. The change in aerobic capacity ($\dot{V}O_2max$) was as follows: 0.2 ± 1.3 mL/kg/min in the controls, −0.04 ± 1.1 mL/kg/min in the fish group, 2.9 ± 1.2 mL/kg/min in the moderate exercise group, and 3.0 ± 2.5 mL/kg/min in the fish + moderate exercise group.

Table 1. Clinical Characteristics of the Groups at Baseline

Characteristic	Control	Fish	Moderate Exercise	Fish + Moderate Exercise
No. of subjects	12	12	11	14
Gender (male/female)	9/3	10/2	8/3	10/4
Age (yr)	53.0 ± 2.0	54.1 ± 2.4	52.3 ± 2.5	52.6 ± 1.9
BMI (kg/m²)	29.7 ± 1.2	29.8 ± 1.3	29.1 ± 0.7	29.9 ± 0.8
Duration of diabetes (yr)	4.4 ± 1.2	3.7 ± 0.9	3.8 ± 1.0	6.8 ± 1.4
Treatment regimen (n)				
Diet only	4	4	3	3
Oral hypoglycemics				
Sulfonylureas	3	4	4	4
Biguanides	2	2	4	3
Combination	3	2	0	4

NOTE. Values are the mean ± SEM. Baseline measures were compared by 1-way ANOVA and were not significantly different.

Table 2. Changes in Platelet Phospholipid Fatty Acids From Baseline to End of Intervention

Fatty Acid	Control (n = 14)	Fish (n = 12)	Moderate Exercise (n = 11)	Fish + Moderate Exercise (n = 12)
20:4ω6	0.81 ± 0.29	−4.26 ± 0.61	0.88 ± 0.53	−2.98 ± 0.70
Total ω6*	1.09 ± 0.41	−5.81 ± 1.01	1.19 ± 0.50	−3.77 ± 0.96
20:5ω3	−0.18 ± 0.07	3.04 ± 0.48	−0.18 ± 0.04	1.69 ± 0.39
22:6ω3	−0.29 ± 0.10	2.00 ± 0.23	−0.43 ± 0.12	1.50 ± 0.21
Total ω3*	−0.48 ± 0.12	5.53 ± 0.71	−0.63 ± 0.10	3.19 ± 0.60

NOTE. Values are the mean ± SEM. Fatty acid changes are given as a percentage of total fatty acids.

*Total ω6 fatty acids (18:2ω6, 20:3ω6, 20:4ω6, and 22:4ω6) and total ω3 fatty acids (20:5ω3, 22:5ω3, and 22:6ω3). For fish main effect on all fatty acids, $P < .0001$.

Platelet Phospholipid Fatty Acids

The fatty acid composition of plasma, platelet, and red blood cell phospholipids at baseline was similar in all groups (data not shown). The changes (expressed as a percentage of total fatty acids) in platelet phospholipid arachidonic acid (20:4ω6), total ω6 fatty acids (sum of 18:2ω6, 20:3ω6, 20:4ω6, and 22:4ω6), eicosapentaenoic acid (20:5ω3), docosahexaenoic acid (22:6ω3), and total ω3 fatty acids (sum of 20:5ω3, 22:5ω3, and 22:6ω3) from baseline to the end of the intervention are shown in Table 2, and indicate compliance with the regular fish intake in the fish-eating groups. The addition of fish to the diet significantly reduced the percentage composition of 20:4ω6 ($P < .0001$) and total ω6 ($P < .0001$) fatty acids, and led to an increase in 20:5ω3 ($P < .0001$), 22:6ω3 ($P < .0001$), and total ω3 ($P < .0001$) fatty acids. The changes in plasma and red blood cell phospholipid fatty acids were comparable to those in platelets (data not shown).

Urinary F_2-Isoprostanes

The mean urinary F_2-isoprostane excretion at baseline in this NIDDM population was 4,131 ± 239 pmol/24 h, with no significant differences between intervention groups (Table 3). There was no significant difference in urinary F_2-isoprostane excretion ($P = .482$) between males (3,836 ± 456 pmol/24 h) and females (4,229 ± 282 pmol/24 h) at baseline. The changes in F_2-isoprostane excretion from baseline to postintervention for each patient in the four groups are shown in a scatter plot in Fig 1, and the changes in group means are also summarized in Fig 1. Despite a variable individual response, there was little change in mean F_2-isoprostane levels in the control group (−68 ± 263 pmol/24 h) and the moderate exercise group (−183 ± 448 pmol/24 h). The fish and fish + moderate exercise groups showed a reduction in F_2-isoprostanes of 759 ± 357 and 1,164 ± 306 pmol/24 h, respectively. There were no significant differences between the fish and fish + moderate exercise groups.

In the GLM, fish diets were significantly associated with a reduction in urinary F_2-isoprostanes of 830 ± 321 pmol/24 h ($P = .013$) after adjusting for baseline values, representing a decrease of 20% (Table 3). This effect was independent of age, gender, and body weight change, yielding an adjusted mean reduction of 833 ± 321 pmol/24 h ($P = .012$) following the fish

Table 3. Urinary F_2-Isoprostane Excretion at Baseline and Postintervention

F_2-Isoprostane Excretion (pmol/24 h)	Control (n = 14)	Fish (n = 12)	Moderate Exercise (n = 11)	Fish + Moderate Exercise (n = 12)	ANOVA Main Effects (*P*)	
					Fish	Exercise
Baseline	4,333 ± 665	4,186 ± 439	3,912 ± 494	4,097 ± 383	NS	
Postintervention*	4,122 ± 334	3,389 ± 319	3,883 ± 334	2,957 ± 296	−830 ± 321 (.013)	−335 ± 323 (.304)
Postintervention†	4,168 ± 331	3,395 ± 317	3,841 ± 332	2,949 ± 292	−833 ± 321 (.012)	−386 ± 318 (.236)

NOTE. Values are the mean ± SEM. Baseline measures were compared by 1-way ANOVA. GLM analysis was used to test for main effects and interactions on postintervention values adjusted for *baseline value and †age, gender, baseline value, and ΔBMI. There were no significant interactions for postintervention F_2-isoprostanes.

Abbreviation: NS, not significant.

diets. Moderate exercise training did not significantly alter F_2-isoprostanes (−335 ± 323 pmol/24 h, $P = .304$). Relative to the light exercise control group and after adjusting for baseline values, postintervention F_2-isoprostane levels were reduced by 733 ± 319 pmol/24 h with the fish diet alone, 239 ± 334 pmol/24 h with moderate exercise alone, and 1,165 ± 296 pmol/24 h with a combination of fish and moderate exercise. The results remained significant for fish-induced changes when F_2-isoprostane values were adjusted for urinary creatinine excretion. F_3-isoprostanes were detected in trace amounts only in several patients taking fish diets.

Correlations Between Urinary F_2-Isoprostanes and Clinical and Biochemical Indices

For all patients combined, baseline F_2-isoprostane levels were significantly correlated with the BMI at baseline ($r = .52$, $P = .0001$). At baseline, there were no significant correlations between F_2-isoprostanes and aerobic fitness, ie, $\dot{V}O_2$max ($r = -.004$, $P = .98$), duration of diabetes ($r = -.09$, $P = .54$), indices of diabetic control (fasting glucose, $r = .13$, $P = .37$; HbA_{1c}, $r = .05$, $P = .72$), or serum lipids (total cholesterol, $r = .04$, $P = .76$; HDL-C, $r = .12$, $P = .43$). Fasting serum triglycerides were significantly associated with F_2-isoprostane levels at baseline ($r = .34$, $P = .02$). There were no significant correlations between baseline levels of F_2-isoprostanes and 20:4ω6 in plasma ($r = .14$, $P = .34$), platelets ($r = .01$, $P = .98$), or red blood cells ($r = .23$, $P = .12$). Similarly, platelet total ω6 fatty acids ($r = -.01$, $P = .94$), 20:5ω3 ($r = -.15$, $P = .31$), 22:6ω3 ($r = -.09$, $P = .57$), and total ω3 fatty acids ($r = -.21$, $P = .16$) were not correlated with F_2-isoprostane levels at baseline. There also were no significant correlations between F_2-isoprostanes at baseline and any of the above-mentioned fatty acids in plasma and red blood cells (data not shown).

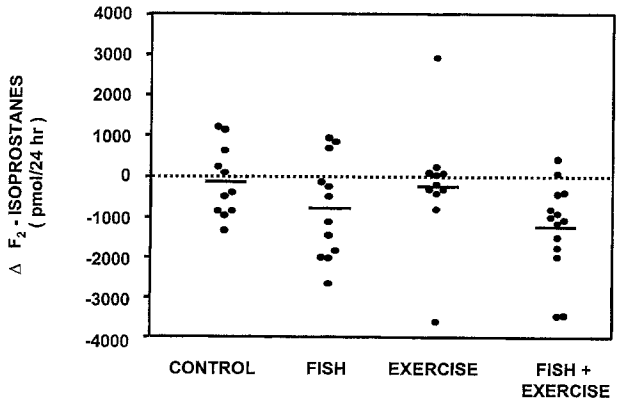

Fig 1. Scatter plot showing changes in urinary F_2-isoprostane excretion from baseline to postintervention for each patient in the 4 groups. Horizontal bars represent the group mean.

Changes in F_2-isoprostanes during the intervention were not significantly correlated with changes in $\dot{V}O_2$max ($r = -.215$, $P = .15$), duration of diabetes ($r = -.17$, $P = .26$), indices of diabetic control (fasting glucose, $r = .16$, $P = .28$, HbA_{1c}, $r = .10$, $P = .50$), or serum lipids (total cholesterol, $r = .08$, $P = .60$; HDL-C, $r = .04$, $P = .79$; triglycerides, $r = .02$, $P = .88$). Changes in urinary F_2-isoprostanes were not significantly correlated with changes in 20:4ω6 (plasma, $r = .24$, $P = .11$; platelets, $r = .10$, $P = .58$; red blood cells, $r = .22$, $P = .18$) or platelet total ω6 fatty acids ($r = .09$, $P = .60$), 20:5ω3 ($r = -.12$, $P = .50$), 22:6ω3 ($r = -.18$, $P = .30$), and total ω3 acids ($r = -.14$, $P = .41$). Changes in plasma and red blood cell fatty acids were not correlated with changes in F_2-isoprostanes (data not shown). Although the BMI at postintervention was significantly correlated with postintervention F_2-isoprostane levels ($r = .37$, $P = .009$), there was no significant association between the change in the BMI and the change in the urinary F_2-isoprostane concentration ($r = .25$, $P = .09$).

DISCUSSION

This randomized controlled study in dyslipidemic NIDDM patients demonstrates that a reduced-fat diet incorporating a daily fish meal rich in ω3 fatty acids substantially reduced in vivo lipid peroxidation by 20% as measured by changes in urinary F_2-isoprostane excretion. A moderate aerobic exercise program had no effect on lipid peroxidation. These results complement our previously reported benefits of a combination of dietary ω3 fatty acids and aerobic exercise on serum lipids and glycemic control in NIDDM patients.[15]

It is well known that diabetics have an increased risk of developing atherosclerosis,[1] and the evidence suggests that free-radical reactions, particularly lipoprotein oxidative modification, may be pivotal pathogenic factors in dictating this increase in risk.[18] One hypothesis is that diabetics are subject to increased postsynthetic chemical modification of lipoproteins by oxidation, glycosylation, or both.[2] Furthermore, most studies have shown that diabetics have reduced antioxidant defenses,[2] which has been interpreted as indicating increased free-radical activity and therefore increased "oxidative stress." Therefore, despite the observation that fish and fish oils rich in ω3 fatty acids may have effects on cardiovascular risk factors that could be of particular benefit to diabetic patients,[3,19] there remains a theoretical concern that they will result in increased lipid

peroxidation.[4] Since the susceptibility of fatty acids to oxidation is proportional to their degree of unsaturation, it has been assumed that increased levels of long-chain ω3 fatty acids would render membranes and lipoproteins, particularly LDL, more susceptible to oxidation and perhaps increase the requirement for antioxidants. However, the data on this issue have been limited and inconclusive.

Studies in vitro have shown that incorporation of ω3 fatty acids in U937 cells and J774 macrophages enhanced lipid radical formation and the capacity of the cells to oxidize LDL.[20,21] In contrast, studies in animals and humans have provided conflicting data. Whereas some studies have shown enhanced lipid peroxidation,[22-28] others have shown no effect or a reduction[29-35] following treatment with ω3 fatty acids. The issue is further complicated by reports of contradictory data within the same studies.[26,29,36,37] It has also been suggested, particularly in the studies showing an increase in lipid peroxidation following treatment with ω3 fatty acids, that an increase in the uptake of these fatty acids may affect the antioxidant status either by impairing the absorption or by causing an increased consumption of antioxidants in plasma and tissues.[28,38,39] Some groups have therefore suggested that ω3 fatty acids should be taken in conjunction with vitamin E supplementation. However, the data from such studies are inconclusive.[22,23,38-42]

The inconsistencies with respect to the effects of ω3 fatty acids and lipid peroxidation could be related to a number of factors, including possible deficiencies in the methodologies used to assess lipid peroxidation. In addition, there could be an adaptive effect whereby enhanced lipid peroxidation may be found more often after short-term compared with long-term supplementation.[29] The types of patients, the quantity and presentation of ω3 fatty acids, and other unidentified constituents of fish oils that could potentially contribute to antioxidant activity may also explain some of the conflicting results. Furthermore, the concentration of the polyunsaturates may be a more important factor affecting lipid peroxidation than the degree of unsaturation.[29] That is, as long as the concentration of ω3 fatty acids increases at the expense of ω6 fatty acids, the susceptibility to oxidation may be unchanged despite an increase in unsaturation.

The measurement of oxidative stress is hampered by methodological problems associated with the measurement of highly reactive species.[43,44] In the case of lipid peroxides, various methods are available but none are entirely satisfactory.[43,44] The most common method measures thiobarbituric acid–reactive substances (TBARS) by a colorimetric assay, but the results must be interpreted with caution.[43,44] The measurement of TBARS is nonspecific and is not considered a good index of the whole-body lipid peroxidation status.[43,44] Although several studies have shown elevated levels of TBARS in humans supplemented with ω3 fatty acids, the results could be attributable to the presence in foods of lipid peroxidation products formed before consumption[45] and/or to inadequate precautions to prevent artifactual lipid peroxidation during the assay.[22,40,41] Other methods for assessing oxidative stress include the assay of conjugated dienes ex vivo in LDL,[46] electron-spin resonance to detect actual free-radical species,[43,44] immunological techniques to measure antibodies to oxidized LDL,[47] and breath excretion of ethane and pentane.[43,44] However, the use of the latter measurement has produced variable results when ω3 fatty acids have been supplemented in animals[42] and humans.[23] Most of the above-mentioned methods represent different aspects of lipid oxidation and collectively provide some knowledge of oxidative damage. However, it may be more important to measure specific markers of particular oxidation reactions.

In this regard, Morrow and Roberts[14] have reported the discovery of a series of chemically stable PGF_2-like compounds (F_2-isoprostanes) that are produced in vivo as products of the free-radical–catalyzed peroxidation of unsaturated fatty acids, primarily arachidonic acid, in membrane phospholipids. The formation of F_2-isoprostanes occurs primarily but not exclusively by a mechanism independent of the cyclooxygenase enzyme. There is substantial evidence that quantitation of F_2-isoprostanes represents a reliable and useful assessment of lipid peroxidation and oxidant stress in vivo.[14] In support of this, elevated levels of F_2-isoprostanes have been reported in animal models of free-radical injury, in human conditions associated with increased oxidant stress, including NIDDM,[48] and in in vitro experimental models.[14] We have established a method for the measurement of urinary F_2-isoprostanes[16] and applied this to the present study to address the question of whether the oxidative status in NIDDM patients is affected by dietary ω3 fatty acids and/or exercise.

Contrary to our hypothesis, urinary F_2-isoprostane levels were significantly reduced following dietary fish supplementation. To our knowledge, this is the first report of F_2-isoprostane changes following ω3 fatty acid supplementation in humans. We also demonstrated no association between the change in urinary F_2-isoprostanes and the increase in ω3 fatty acids or the decrease in ω6 fatty acids in plasma, platelets, and red blood cells. This lack of association with the changes in fatty acids is important in view of the fact that F_2-isoprostanes are predominantly derived from the peroxidation of arachidonic acid, which, along with other ω6 fatty acids, was reduced following the fish diets. The results therefore reflect a true reduction in the oxidative status rather than a change resulting from a reduced supply of substrate.

It remains unclear as to how ω3 fatty acids reduced F_2-isoprostane excretion. However, it may be related to the antiinflammatory actions of ω3 fatty acids and the expected reduction in leukocyte activity. There is evidence that ω3 fatty acids reduce the leukocyte "oxidative burst."[49-51] A decreased superoxide production by isolated human polymorphonuclear leukocytes[52] and monocytes[49,53] and by rat polymorphonuclear leukocytes[54] has been reported after dietary treatment with ω3 fats. Other mechanisms by which ω3 fatty acids may protect against lipid peroxidation could be related to their tight packing in complex membrane lipids and lipoproteins making the double bonds less available for free-radical attack,[55] inhibition of the activity of phospholipase A_2, a prooxidant enzyme,[56] and induction of the activity of hepatic antioxidant enzymes such as glutathione peroxidase.[57]

The measurement of urinary F_2-isoprostanes as an in vivo marker of free-radical–mediated oxidation in exercising subjects is another unique aspect of the present study. The observation of unchanged F_2-isoprostane levels following mod-

erate exercise training is an important finding, particularly since many recent studies have demonstrated that even moderate-intensity exercise may increase lipid peroxidation and oxidative stress.[7-9] For example, Laaksonen et al,[9] using a similar mode of training and exercise intensity as the present study, observed a substantial increase in plasma TBARS immediately after a 40-minute acute bout of exercise at 60% $\dot{V}O_2$max in healthy type 1 diabetic men. The difference in the findings may reflect an adaptive response to regularly performed moderate exercise. Indeed, several recent studies in both animals and humans have indicated that regular aerobic exercise can improve the body's antioxidant systems[10,11] and may subsequently reduce resting and exercise-induced oxidative stress.[12,13] However, differences in the mode of exercise used, the intensity and duration of the activity, the subject characteristics, and the methods for measuring exercise-induced lipid peroxidation have previously made it difficult to draw definitive conclusions.

We have found improved aerobic fitness and glycemic control following moderate exercise training in these patients with NIDDM.[15] However, in contrast to previous findings,[9] we did not observe a relationship between $\dot{V}O_2$max and F_2-isoprostanes at baseline, and the improvement in cardiorespiratory fitness with moderate exercise was not related to F_2-isoprostane levels. It is probable that the uniformly lower baseline $\dot{V}O_2$max in our subjects in comparison to healthy type 1 diabetics[9] may account for this discrepancy in findings. While the impact of longer-term training and larger cardiovascular fitness changes on oxidative stress will require further investigation, our findings show that, at least in the short-term, patients with NIDDM can benefit from improved cardiorespiratory fitness and glycemic control with regular moderate exercise without adverse effects on oxidative stress.

In conclusion, this is the first report assessing the independent and combined effects of dietary ω3 fatty acids and aerobic exercise on lipid peroxidation. The study is unique in that we used the measurement of urinary F_2-isoprostanes, which is believed to be representative of whole-body in vivo lipid peroxidation. We have shown that in NIDDM, there is a strong association between baseline urinary F_2-isoprostanes and the BMI. In contrast to previous reports, lipid peroxidation as measured by urinary F_2-isoprostanes was unchanged by the moderate exercise program and was reduced by dietary ω3 fatty acids. These findings suggest that the daily intake of fish in combination with a moderate exercise program does not lead to adverse effects on lipid peroxidation and could complement the known benefits of both modalities on serum lipids and glycemic control, favoring an overall reduction in the cardiovascular risk in diabetic patients.

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