EPA and 600 mg DHA). e so gels, which contained 4 I.U. Vitamin E, were provided by the All Nature Pharmaceuticals, Inc, USA. ere were only negligible amounts of gelatin, glycerin and puri ed water. Compliance was assessed by so gels counts and periodic telephone or personal contact.

Nutritional methods and lifestyle assessment

Before the baseline period, a dietician gave verbal instructions to the subjects on how to keep accurate dietary records, including how to weigh or measure foods. Six days of dietary data were collected at each interview (four 24-hour recalls at the interview and two days of food records that had been kept before the interview) and a lifestyle questionnaire including history of illness, supplements intake, medications, anthropometric measurements, demographic information and physical activity were completed at baseline, week 4 and week 8. Waist-to-hip ratio was calculated as the body circumference midway between the inferior border of the rib cage and the superior border of the iliac crest, divided by the maximal body circumference at the buttocks [9]. e Nutritionist IV (version 4; N-Squared Computing, San Bruno, CA) computer program was used to transform data and calculate EPA and DHA intake and to determine mean daily nutrient intakes**.** Weight, dietary intake, changes in physical activity and medication, and any illness were recorded each week during baseline and at weeks 4, and 8 of the intervention. Blood pressure was measured to within 2 mm Hg, with the patient semirecumbent a er resting for ve minutes, using a mercury sphygmomanometer.

Blood biochemistry

Serum glucose was determined according to the glucose oxidase method with an autoanalyzer (Beckman Instruments). Serum insulin concentrations were determined with ELISA (Monobind Inc, USA). Cholesterol and triacylglycerol concentrations were measured a er an overnight fast by the CHOP-PAP method and GPO-PAP method respectively (both supplied by Roche Diagnostics, Lewes, UK). HDL cholesterol concentrations were measured a er precipitation with phostungstic acid/Mg^{2+} (Roche Diagnostics), while LDL cholesterol was calculated as follows: LDL=TG–(HDL + TG/5) [10]. QUIKI was assessed as: 1/[log fasting insulin (m U/ml)+log fasting glucose (mg/100 ml)] [11].

Analysis of acute-phase proteins and cytokines

Serum samples for cytokine concentrations were stored at -70°C until assay. Serum concentrations of TNF−α, IL-1 β, IL-6, and CRP were determined in duplicate by Instant ELISA (Bender MedSystems, Vienna, Austria and DCB-Diagnostics Biochem Canada Inc, for CRP). All instant ELISAs were established to meet the following criteria: linearity of signal for the standard curve between Optical Density (OD) 0.05 and 2.0 , di erence between expected and measured signal in spiking experiments less than 10%, mean intra-assay variation below 10%, mean interassay variation below 10%, loss of signal a er freezing and thawing of sera three times less than 10%. Dilution curves of serum samples were parallel those of standard. Intra-assay and interassay coe cients of variations were 5.3% and 4.2%, respectively, for TNF−α; 5.1% and 4.3%, respectively, for IL-1 β; 4.2% and 3.2%, respectively for IL-6 and 5.1% and 3.1%, respectively, for CRP.

Serum sialic acid determination was performed as previously described [12]. Samples from baseline, week 4, and at the end of the intervention were measured in a single assay to minimize interassay variation.

Statistical analysis

Data were analysed by using SPSS version 12.0 (SPSS Inc, Chicago). e normality of all variables was assessed by examining their histograms and using Kolmogorov-Smirnov test. No signi cant deviation from normality was observed among data either before or a er the intervention. Signi cance levels were adjusted for multiple comparisons by using the Bonferroni method. All values are reported as means ± SEs except for the characteristics of the patients at baseline, which are reported as means ± SDs. Repeated-measures analysis of variance comparing the variables between two sexes and di erent time points of the intervention were performed using SYSTAT 10 (SPSS Inc, Chicago). All tests were two-tailed and P-values less than 0.05 were taken as signi cant. e correlations between variables were examined by Pearson correlation linear regression, by using the SYSTAT program.

Results

Study population

All subjects completed the study. e characteristics of the patients are shown in Table 1. None of the subjects was taking antihypertensive medications or oral hypoglycemic agents.

Diet, lifestyle, and blood pressure

ere were no signi cant di erences in total energy intake, EPA/DHA intake, polyunsaturated to saturated fatty acid (P:S) ratio, and macronutrient intake at baseline, week 4 and week 8 in the control and the intervention group (data not shown). Alcohol intake and pw >>BDC 0 Tw T*(m)4(e)-5(d2es i (p)16(19(ts.)]To)12(f)1(t))1(2w)-3 i(a)-

Fasting glucose (mmol/L)	
Baseline	9.56 ± 0.63
Week 4	10.02 ± 0.64
Week 8	9.28 ± 0.66
Fasting insulin (pg/ml)	
Baseline	58.75 ± 9.60
Week 4	67.55 ± 10.90
Week 8	77.90 ± 17.00
Fasting insulin/glucose*	
Baseline	

in fasting serum glucose, insulin concentrations and QUIKI at baseline week 4, and week 8 (data not shown). ere were no signi cant changes in fasting serum glucose, insulin, and QUIKI at week 4 and at the end of intervention compared to baseline (Table 2).

Serum lipids

e mean values for serum lipids at baseline, week 4, and week 8 are shown in Table 3. ere were no signi cant di erences between males and females in fasting serum lipids (cholesterol, triacylglycerol, HDL-C, LDL-C, LDL-C/HDL-C, and Chol/HDL-C) at baseline, week 4 , and week 8 (data not shown). At the end of intervention, fasting cholesterol, and triacylglycerol, concentrations decreased by 6% ($P<0.02$), and 19% ($P<0.009$), respectively. LDL-C to HDL-C ratio, and Chol to HDL-C ratio decreased by 4% (NS), and 6% ($P<0.001$), respectively. ere were no signi cant changes in total HDL, and LDL cholesterol concentrations, at the end of the intervention (Table 3).

Serum cytokines

ere were no signi cant di erences between males and females in serum IL-6, IL-1 β, TNF−α, CRP, and sialic acid concentrations at baseline, week 4, and week 8 (data not shown).

ere were no signi cant changes in serum TNF−α, or IL-1 β concentrations following 8 weeks EPA/DHA supplementation, compared to baseline (Table 4). ere was a no signi cant trend for TNF−α to be increased following EPA/DHA supplementation at week 4 and 8 compared to baseline.

Mean serum sialic acid concentrations at week 8 were signi cantly lower than baseline value. Serum IL-6 concentration showed a signi cant decrease at week 4 but showed a no signi cant increase at the end of intervention; mean concentrations were still lower than baseline value. At week 8, there was a 22% signi cant decrease in CRP concentrations comparing week 4 and baseline value but this was not signi cant. Serum IL-1 β showed a no signi cant 29% decrease at week 4 and returned to baseline value at week 8 (Table 4).

Associations between glycemia indices, lipids and in ammatory markers

For all patients combined, there was no signi cant correlation between glycemia indices (fasting glucose and insulin) with

in ammatory markers (IL-6, IL-1 β, TNF−α, CRP, and sialic acid) at baseline.

ere were no signi cant correlations between serum lipids and markers of in ammation at baseline.

ere was no signi cant correlation between markers of in ammation (IL-6, IL-1 β, TNF−α, CRP, and sialic acid) with duration of diabetes at baseline. Table 5 shows the linear regression analysis controlled for age, gender and BMI, which explains 32.0 % of variation in QUIKI. Only IL-6 remained in the model during the stepwise analysis. IL-6 seemed to play a main role in in uencing QUIKI, while IL-1β, CRP, and TNF-α did not play any signi cant role. A er exclusion of IL-6 from the group of independent variables, the value of R square decreased by 25% (data not shown), which could re ect interferyout >>BDCe

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