Lower Serum Non-Esterified Eicosapentaenoic Acid (EPA) is Associated with Insulin Resistance: PROspective Metabolism and ISlet Cell Evaluation (PROMISE) Cohort

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Introduction

- Elevated **total** non-esterified (i.e. free) fatty acids (NEFA) are associated with greater risk for type 2 diabetes[1, 2]. NEFA may contribute to insulin resistance and β-cell dysfunction through lipotoxicity and inflammation[3].
- However, NEFA are a **diverse** class of molecules, varying in biochemical action depending on chain length and unsaturation[4].
- Specifically, the n-3 long chain polyunsaturated fatty acid (LC-PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may modulate the pathology of diabetes via several potential mechanisms (e.g. anti-inflammation and anti-lipotoxicity) [5].
- To date, no study has examined the relationship between n-3 **non-esterified** LC-PUFA and the pathophysiology underlying diabetes.

Objectives & hypothesis

- **Objective:** To determine the cross-sectional association of serum non-esterified EPA, docosapentaenoic acid (DPA), DHA, and total n-3 LC-PUFA on insulin resistance and β-cell function in a population at-risk for type 2 diabetes.
- **Hypothesis:** Given the potential anti-inflammatory and anti-lipotoxic properties of long-chain n-3 PUFA, we hypothesize that greater levels of either EPA, DPA, DHA, or total n-3 LC-PUFA will be associated with lower insulin resistance and greater β-cell function.

Methods

- Data were used from the baseline visit (2004–2006) of the observational PROspective Metabolism and ISlet cell Evaluation (PROMISE) cohort. Subjects were recruited from Toronto and London, Ontario, Canada and at recruitment had $\geq$ 1 risk factors for diabetes. Analytic sample was 476 participants.
- An 8–12hr fasting oral glucose tolerance test (OGTT) was conducted (blood samples at 0, 30, and 120 min).
- HOMA-IR and the Matsuda Insulin Sensitivity Index (ISI) were calculated for insulin resistance. Insulogenic Index over HOMA-IR (IGI/IR) and Insulin Secretion Sensitivity Index 2 (ISSI-2) were calculated for β-cell function.
- All measures were validated against gold standard tests.
- Serum NEFA were determine using thin-layer chromatography with gas chromatography coupled with flame ionization detector.
- Anthropometrics measured (BMI, waist circumference (WC)-to-height ratio).
- Questionnaires assessed sociodemographics and lifestyle variables.
- Cross-sectional multiple linear regression analysis was conducted, adjusted for confounders selected based on the causal directed acyclic graph approach.
- EPA, DPA, DHA, and total n-3 LC-PUFA are shown as a percentage of total NEFA in regression models.

Results: Basic characteristics of study participants

Basic characteristics of participants from the PROMISE cohort at the baseline visit (2004–2006).

(a)

	Mean (SD)
Age (yrs)	50.59 (9.76)
BMI (kg/m^2)	31.15 (6.45)
WC (cm)	98.49 (15.49)
FI (pmol/L)	75.18 (56.25)
FG (mmol/L)	4.95 (0.53)
gAUC	13.73 (2.27)
iAUC	870.18 (552.79)

(b)

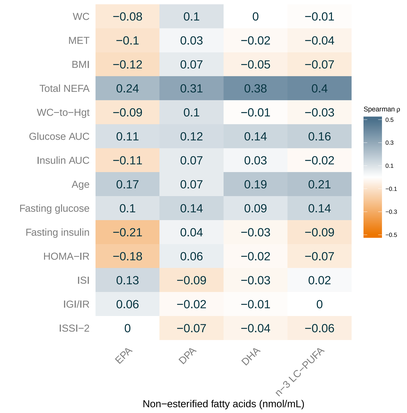
	Median (IGR)
NEFA* (nmol/mL)	383.45 (116.52)
EPA (nmol/mL)	0.54 (0.38-0.83)
DPA (nmol/mL)	0.54 (0.39-0.78)
DHA (nmol/mL)	1.47 (1.04-2.09)
n-3 LC-PUFA (nmol/mL)	2.72 (1.99-3.61)
HOMA-IR	1.82 (1.17-3.06)
ISI	5.46 (3.47-8.71)
IGI/IR	9.55 (5.46-14.94)
ISSI-2	727.37 (570.03-919.61)

(c)

	N (%)
Other Dis	273 (57.7)
Caucasian	334 (70.8)
Hispanic	55 (11.7)
Other	51 (10.8)
South Asian	32 (6.8)
Female	347 (73.5)
Inactive	371 (77.9)

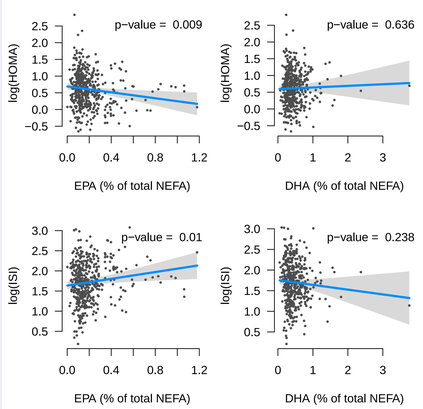
Note: * The NEFA variable value is mean (SD). WC = waist circumference; FI = fasting insulin; FG = fasting glucose; gAUC = glucose area-under-the-curve; iAUC = insulin area-under-the-curve; Other Dis = Presence of other chronic diseases, e.g. hypertension, stroke. Other = Ethnicities other than the three mentioned. N = 455–476.

Results: Correlations between n-3 LC-PUFA and basic characteristics



Correlation heatmap of non-esterified EPA, DPA, DHA, and total n-3 LC-PUFA with anthropometric and metabolic variables from the PROMISE cohort (2004–2006).

Results: Partial residual plots of the fully adjusted (model 2) linear regression analyses



Partial residual plots of the fully adjusted (Model 2) association of non-esterified EPA and DHA (as a percent of total NEFA) with HOMA-IR and ISI.

Results: Linear regression analyses

Multiple linear regression models of non-esterified EPA, DHA, and n3 LC-PUFA with insulin sensitivity and β-cell function measures from PROMISE subjects without diabetes at the baseline visit (2004–2006), adjusted for covariates.

	EPA		DHA		n-3 PUFA	
	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*
HOMA-IR						
Model 1	**-0.43 (0.16)**	**0.008**	0.01 (0.08)	0.894	-0.03 (0.06)	0.582
Model 2	**-0.44 (0.17)**	**0.009**	0.05 (0.1)	0.636	-0.02 (0.07)	0.764
ISI						
Model 1	**0.38 (0.16)**	**0.015**	-0.08 (0.08)	0.296	-0.01 (0.05)	0.899
Model 2	**0.42 (0.16)**	**0.010**	-0.12 (0.1)	0.238	-0.01 (0.07)	0.918
IGI/IR						
Model 1	0.24 (0.24)	0.305	0.12 (0.12)	0.306	0.11 (0.08)	0.188
Model 2	0.12 (0.25)	0.627	0.04 (0.15)	0.801	0.05 (0.1)	0.617
ISSI-2						
Model 1	0.06 (0.1)	0.537	0.05 (0.05)	0.300	0.04 (0.04)	0.230
Model 2	0.01 (0.11)	0.895	0.02 (0.06)	0.720	0.02 (0.05)	0.636

Note: DPA had similar non-significant associations as DHA. n=454–466. HOMA-IR, ISI, IGI/IR, and ISSI-2 were log transformed. The n-3 NEFA are as a percent of total NEFA. Model 1: Age, sex, ethnicity, and WC-to-height ratio. Model 2: Model 1 + presence of other chronic diseases (i.e. hypertension, cancer, myocardial infarction, or stroke), n-6 LC-PUFA, and physical activity (MET).

Conclusions

- Greater proportion of EPA in the non-esterified pool was associated with lower insulin resistance, but not with β-cell function.
- No significant association of DPA, DHA, nor n-3 LC-PUFA on insulin resistance or β-cell function.
- Results suggest that EPA may be involved with both hepatic and peripheral insulin sensitivity, potentially by protecting against lipotoxicity of insulin sensitive tissues.
- Limitations include the cross-sectional analysis and use of proxy measures for the outcomes.

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