Serum Non-Esterified Fatty Acid (NEFA) Concentrations are Associated with Longitudinal Progression of Beta-Cell Dysfunction: Prospective Metabolism and Islet Cell Evaluation (PROMISE) Cohort

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Background

- Elevated NEFA concentrations are associated with incident type 2 diabetes (T2DM)[1–3], however NEFA are comprised of physiologically diverse species.
- Limited longitudinal data exist on total and individual NEFA in the progression of insulin sensitivity and beta-cell function.
- Our **objective** was to study the longitudinal associations of NEFA concentrations and individual NEFA species with 6-yr changes in insulin sensitivity (IS) and beta-cell function.

Methods: PROMISE cohort

- Adults at-risk for diabetes were recruited from Toronto and London, ON and followed every 3-yrs.
- Participants completed an OGTT at each visit with 3 blood samples (0, 30, 120 min), used to measure glucose, insulin, and fasting NEFA (n=478), in addition to standard clinical measures.
- NEFA species were quantified at baseline using thin layer chromatography and gas liquid chromatography coupled to flame ionization detector.
- From glucose and insulin values, the following were computed:
  - IS: 1/HOMA-IR[4] and ISI (Matsuda Index)[5]
  - Beta-cell function: Insulinogenic index[6] over HOMA-IR (IGI/IR) and Insulin Secretion-Sensitivity Index-2 (ISSI-2)[7]
- Dysglycemia status was assigned if the participant had incident impaired fasting glucose, impaired glucose tolerance, or diabetes at either of the two follow-up visits.
- Statistical analysis involved generalized estimating equations adjusting for waist circumference, physical activity (MET), alcohol intake, and sex.

Results: Basic characteristics

Table 1:Basic characteristics of PROMISE participants at each clinic visit over the 6 years. All values shown are mean (SD), median (IQR), or in n (%), with *p*-value from an ANOVA (outcome variables were log-transformed) or a chi-square test.

Measure	0-yr	3-yr	6-yr	p
n	461	445	366	
BMI (kg/m^2)	31.14 (6.44)	31.43 (6.51)	31.11 (6.59)	
WC (cm)	98.53 (15.46)	99.31 (15.71)	100.45 (15.74)	
Age (yrs)	50.59 (9.75)	53.8 (9.7)	56.87 (9.6)	*
MET	25.53 (53.16)	25.47 (50.02)	22.35 (48.52)	
Chol (mmol/L)	5.17 (0.93)	5.13 (0.99)	5.11 (0.88)	
TAG (mmol/L)	1.48 (0.77)	1.45 (0.84)	1.45 (0.66)	
LDL (mmol/L)	3.13 (0.83)	3.15 (0.85)	3.09 (0.78)	
HDL (mmol/L)	1.37 (0.38)	1.32 (0.35)	1.36 (0.42)	
HOMA-IR	1.82 (1.18-3.06)	2.26 (1.39-3.76)	2.32 (1.51-3.63)	*
ISI	5.46 (3.5-8.68)	4.65 (2.75-7.65)	4.62 (2.98-6.97)	*
IGI/IR	9.55 (5.46-14.94)	7.37 (4.46-13.85)	7.14 (4.35-12.09)	*
ISSI-2	727.25 (569.58-918.6)	613.44 (493.85-836.72)	622.27 (471.81-806.02)	*
Caucasian	335 (70%)	337 (71%)	333 (71%)	
Hispanic	55 (12%)	55 (12%)	52 (11%)	
Other ethnicity	51 (11%)	51 (11%)	50 (11%)	
South Asian	32 (7%)	32 (7%)	31 (7%)	
Female	347 (73%)	349 (73%)	293 (62%)	
IFG	10 (2%)	14 (3%)	14 (3%)	
IGT	13 (3%)	82 (17%)	62 (13%)	*
DM	3 (1%)	29 (6%)	24 (5%)	*

Note: WC = waist circumference, Chol = cholesterol, MET = Metabolic Equivalents (physical activity). The "n" sample size represents the number of participants with ISSI-2 data. Variables do not all add up to the same sample size across years, as some variables contain missingness. * p<0.05.

Results: Brief overview

- Over the 6-yr period, the outcomes decreased between 14.4% to 27.5% while estimates of adiposity (BMI and waist circumference) did not change (<2%).
- Of the NEFA species, palmitic acid, oleic acid, and linoleic acid comprised the majority (74.76%) of the pool.
- Consistent with previous research, total NEFA was associated with a higher risk for dysglycemia over the 6-yrs (RR = 1.25 (95% CI 1.05 to 1.43) for every SD increase in total NEFA).
- NEFA had low correlations with all basic characteristics (Fig. 3).
- From the GEE analysis (Fig. 4), few species were associated with IS; stronger associations were seen with beta-cell function.
- Palmitic acid, oleic acid, linoleic acid, and alpha-linolenic acid were strongly associated with declines in beta-cell function (Fig. 4).

Results: Time plots of outcomes and estimated adiposity

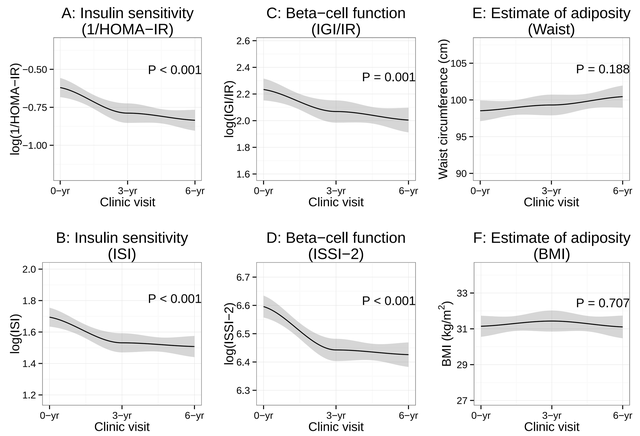

Figure 1:Longitudinal trends in the outcome variables and estimates of adiposity over 6-yrs in PROMISE participants. Lines represent a smoothing regression line (LOESS: localized regression) and the shaded area is the 95% CI, with the p-value calculated from an ANOVA.

Results: Distribution of NEFA

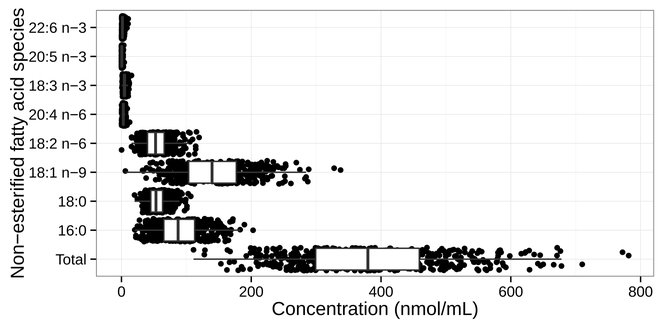

Figure 2:Distribution of non-esterified fatty acid species in absolute concentrations. Dots represent individual data points, while the boxes represent the median and interquartile range.

Results: Spearman correlation heatmap

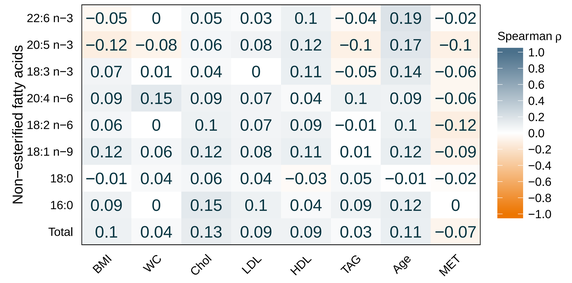

Figure 3:Spearman correlation heatmap of NEFA species and basic characteristics.

Results: GEE forest plot

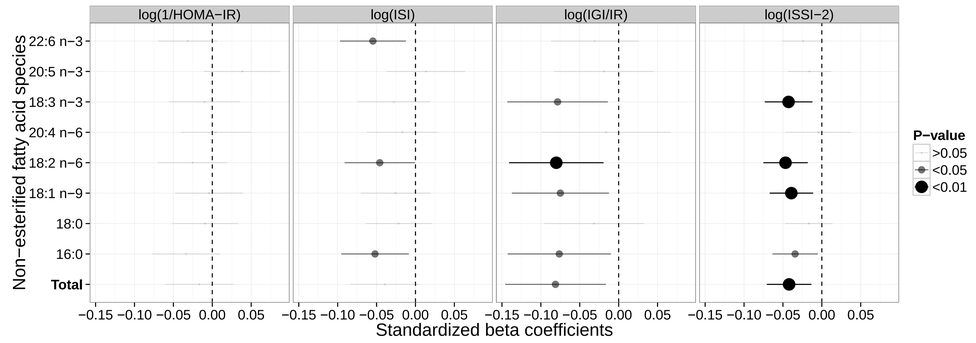

Figure 4:Longitudinal associations of non-esterified fatty acids with insulin sensitivity and beta-cell function over a 6-yr period. Beta coefficients from the generalized estimating equations analysis (dots) have been standardized to compare across across NEFA species, with larger dots representing stronger associations. Lines represent the 95% confidence interval. The dashed line represents the null line. Negative beta values represent declines in the outcomes. Covariates: Sex, waist size, alcohol intake, and physical activity. Interpretation e.g.: For every 1 SD increase in total NEFA, there is a 5% decrease in ISSI-2.

Discussion

- Species that associated strongly with beta-cell function decline also tended to comprise the majority of the NEFA pool, suggesting **total NEFA** mainly drove the associations seen.
- Our findings extend the current literature by showing a strong association of NEFA with beta-cell function decline, which is consistent with literature suggesting that beta-cells are particularly sensitive to lipotoxicity[2].

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