



n-3 Fatty Acids Attenuate the Risk of Diabetes Associated With Elevated Serum Nonesterified Fatty Acids: The Multi-Ethnic Study of Atherosclerosis

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OBJECTIVE

Chronically high nonesterified fatty acids (NEFAs) are a marker of metabolic dysfunction and likely increase risk of type 2 diabetes. By comparison, n-3 fatty acids (FAs) have been shown to have various health benefits and may protect against disease development. In 5,697 participants of the Multi-Ethnic Study of Atherosclerosis (MESA), we examined whether serum levels of NEFAs relate to risk of incident type 2 diabetes and further tested whether plasma n-3 FA levels may interact with this relation.

RESEARCH DESIGN AND METHODS

NEFAs were measured in fasting serum using an enzymatic colorimetric assay and phospholipid n-3 FAs eicosapentaenoic and docosahexaenoic acids were determined in plasma through gas chromatography-flame ionization detection in 5,697 MESA participants. Cox proportional hazards regression evaluated the association between NEFA levels and incident type 2 diabetes and whether plasma n-3 FAs modified this association adjusting for age, sex, race, education, field center, smoking, and alcohol use.

RESULTS

Over a mean 11.4 years of the study period, higher diabetes incidence was found across successive NEFA quartiles (Q) (hazard ratio [95% CI]): Q1, 1.0; Q2, 1.35 (1.07, 1.71); Q3, 1.58 (1.24, 2.00); and Q4, 1.86 (1.45, 2.38) ($P_{\text{trend}} < 0.001$). A significant interaction of n-3 FAs on the relation between NEFAs and type 2 diabetes was also observed ($P_{\text{interaction}} = 0.03$). For individuals with lower n-3 levels (<75th percentile), a higher risk of type 2 diabetes was observed across quartiles of NEFAs: Q1, 1.0; Q2, 1.41 (1.07, 1.84); Q3, 1.77 (1.35, 2.31); and Q4, 2.18 (1.65, 2.88) ($P_{\text{trend}} < 0.001$). No significant associations were observed in those with n-3 FAs \geq 75th percentile ($P_{\text{trend}} = 0.54$).

CONCLUSIONS

NEFAs are a marker of type 2 diabetes and may have clinical utility for detecting risk of its development. The modifying influence of n-3 FAs suggests a protective effect against disease and/or metabolic dysfunction related to NEFAs and requires further study.

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Type 2 diabetes and insulin resistance (IR) affect some one-third of Americans (1), and their unabated progression results in a host of comorbidities, crippling disability, and premature death (2). Despite these long-term health consequences, the nascent stages of type 2 diabetes have been shown to be reversible (3), making early identification vitally important. Elevated fasting levels of nonesterified fatty acids (NEFAs) may represent an early type 2 diabetes risk marker, but few large prospective studies have examined whether they predict future disease incidence.

Functionally, NEFAs serve as a key energy source for skeletal muscle and myocardium as well as other major organs, but chronically high levels indicate metabolic dysfunction and have been associated with obesity and risk of type 2 diabetes (4–8), though the null finding has also been reported (9). In addition to being a potential marker of pathophysiology, NEFAs have been shown to directly promote IR in liver and skeletal muscle across experimental studies (10–13). Finally, NEFAs have been shown to induce inflammation (14,15), a known suppressor of insulin-mediated glucose uptake and inducer of IR (16–18). Overall, serum NEFA concentrations may not only serve as a marker of type 2 diabetes, but also promote its development.

In contrast to NEFAs, the n-3 fatty acids (FAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to have benefits with respect to metabolic function. n-3 FAs have been found to both stimulate insulin sensitivity (19–22) and reduce inflammation (22–25), which would be expected to halt or suppress the development of type 2 diabetes. Despite such evidence, a protective influence of n-3 FAs has been mixed in prospective study populations with n-3 levels reported to be inversely related (26), positively related (27,28), and unrelated to type 2 diabetes incidence (29,30). Such discrepancies among experimental and prospective studies suggest that high n-3 FAs may not be a strong suppressor of type 2 diabetes, but n-3 FAs may blunt specific forms of metabolic dysfunction such as those related to elevated NEFA levels.

Taken together, there is sufficient evidence showing n-3 FAs and NEFAs have

opposing influences on inflammatory and IR-inducing pathways. As NEFAs have been shown to promote IR and inflammation, it was hypothesized that high circulating NEFA levels will predict type 2 diabetes incidence. In contrast, n-3 FAs have been shown to suppress inflammation and promote insulin sensitivity; therefore, it was hypothesized that high n-3 FAs (EPA + DHA) will modify (i.e., interact with) the NEFA–diabetes association by mitigating disease incidence in those with higher NEFA levels. Given the controversy across studies of dietary n-3 FAs, an objective, direct measurement of plasma n-3 FAs was used to more accurately assess their bioavailability.

RESEARCH DESIGN AND METHODS

Population

The primary aim of MESA is to investigate clinical and subclinical coronary heart disease development and progression. The study design has been previously described (31), and information about MESA is also available at <http://www.mesa-nhlbi.org>. MESA is composed of 6,814 men and women between the ages of 45 and 84 years and without evidence of clinical coronary heart disease at baseline. The population of 38.6% white, 27.6% black, 11.8% Chinese, and 22.0% Hispanic subjects was recruited from six communities in the U.S. (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; New York, NY; and St. Paul, MN). Institutional Review Board approval was obtained at all MESA sites, and all participants gave informed consent. Recruitment and baseline examinations began in July 2000 and were conducted over a 24-month period. Exclusions for this analysis include participants with prevalent diabetes ($n = 680$), those with baseline fasting glucose ≥ 126 mg/dL ($n = 179$), or missing data for plasma phospholipid FAs ($n = 241$) or serum NEFAs ($n = 91$), leaving a total 5,697 participants in this subcohort.

Plasma and Serum Measurements

Twelve-hour fasting blood was drawn, and serum and EDTA-anticoagulant tubes were collected and stored at -70°C using a standardized protocol (31). Serum insulin was measured by the Linco Human Insulin Specific RIA Kit (Linco Research, Inc., St. Charles, MO) and serum glucose by rate reflectance

spectrophotometry using thin-film adaptation of the glucose oxidase method on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY) in a central laboratory (Collaborative Studies Clinical Laboratory at Fairview-University Medical Center, Minneapolis, MN) using Centers for Disease Control and Prevention–standardized methods. C-reactive protein was measured using the BNII nephelometer (N High Sensitivity CRP; Dade Behring Inc, Deerfield, IL). NEFA levels (mmol/L) were determined in serum using a colorimetric assay (HR Series NEFA-HR [2]; Wako Diagnostics, Richmond, VA).

Phospholipid FAs were extracted from EDTA plasma using the chloroform/methanol method previously described by Cao et al. (32). Briefly, lipids are extracted with a mixture of chloroform/methanol (2:1, volume for volume) and subclasses separated using thin-layer chromatography. The phospholipid band is harvested and derivatized to methyl esters. The final product is dissolved in heptane and injected onto a capillary Varian CP7420 100-m column with a Hewlett Packard 5890 gas chromatograph. The gas chromatograph is configured for a single capillary column with a flame ionization detector and interfaced with HP chemstation software. FAs were expressed as a percent of the total phospholipid FAs. The following representative coefficients of variation were obtained ($n = 20$): EPA, 7.2%; and DHA, 3.2%.

Demographic and Anthropometric Characteristics

Information regarding age, sex, race/ethnicity, education, and lifestyle factors was obtained by questionnaires. Height (m), weight (kg), and waist circumference were measured according to standard procedures (31).

Incidence of Type 2 Diabetes

Type 2 diabetes incidence was determined across 5 exams and 12 follow-up contacts over an average of 11.4 years and was defined by one of the following criteria: reported physician diagnosis, use of antidiabetes medications, or fasting (12-h) glucose >126 mg/dL.

Statistical Methods

SAS version 9.3 (SAS Institute; Cary, NC) was used to analyze the data. Baseline characteristics are presented as means

(SE) for continuous variables and frequencies (%) for categorical variables according to quartiles of NEFAs. In addition, baseline characteristics were also presented by n-3 (EPA + DHA) FA category (<75th percentile and ≥75th percentile). Cox proportional hazards regression analysis evaluated the relation of quartiles of NEFAs with the development of type 2 diabetes. Two statistical models were developed: model 1 was adjusted for age, sex, race, education, field center, current smoking, current alcohol intake, and plasma n-3 FAs. As adiposity and inflammation are likely in the pathway(s) of interest, model 2 was adjusted for model 1 plus waist circumference and C-reactive protein. Effect modification of n-3 FAs on this relation was suspected, so an interaction term for n-3 FA levels was included in the statistical models. Plasma n-3 FAs were examined as an interaction variable, dichotomizing levels by <75th percentile of the distribution versus ≥75th percentile of the distribution. Statistical tests were considered significant at $P < 0.05$.

RESULTS

Excluding patients with diabetes at baseline, 657 individuals developed type 2 diabetes over an average 11.4 years of follow-up. Adjusted baseline characteristics stratified by quartiles of NEFAs for 5,697 MESA participants are shown in Table 1. Characteristics across dichotomized plasma n-3 FA categories are shown in Table 2, and significant differences were observed between those with low (<75th percentile) and high (≥75th percentile) n-3 FA levels. A greater proportion of those with elevated n-3 FA levels were older, female, and nonsmokers; had lower BMI and waist circumferences; and had lower levels of fasting insulin and hs-CRP.

For the main effect, hazard ratios (HRs) and 95% CIs for the association between NEFAs and incident type 2 diabetes are reported in Table 3. Significantly higher risks for developing type 2 diabetes were observed across successive NEFA quartiles after adjusting for age, sex, race, education, site, smoking, and alcohol use. The HRs (95% CIs) across quartiles 1–4 were 1.0; 1.35

(1.07, 1.71); 1.58 (1.24, 2.00); and 1.86 (1.45, 2.38), respectively ($P_{\text{trend}} < 0.001$). Further adjustment for waist circumference and hs-CRP attenuated the effect sizes, but associations between NEFAs and incident type 2 diabetes remained significant ($P_{\text{trend}} = 0.006$).

A significant interaction of plasma n-3 FAs (high vs. low levels) on the relation between NEFAs and incident type 2 diabetes ($P_{\text{interaction}} = 0.03$) was found, shown in Table 4. For individuals with n-3 FAs ≥75th percentile of the distribution, no statistically significant relationship between NEFAs and incident type 2 diabetes was found. For individuals with n-3 FAs <75th percentile, their risk of developing type 2 diabetes was significantly greater across successive NEFA quartiles. Further adjustment for waist circumference and hs-CRP attenuated the associations between NEFAs and type 2 diabetes, but the association remained significant across quartiles ($P_{\text{trend}} < 0.001$).

A separate Cox proportional hazards model examining the association between plasma n-3 FAs and risk of type

Table 1—Mean (SE) and frequency (%) of baseline characteristics stratified across quartiles of NEFAs, MESA, $N = 5,697$

Characteristic	Quartiles of NEFAs				P_{trend}
	1 ($n = 1,380$)	2 ($n = 1,461$)	3 ($n = 1,455$)	4 ($n = 1,401$)	
Quartile, n					
NEFA range	0.13–0.39	0.40–0.52	0.53–0.67	0.68–2.11	
Age, years, mean (SE)	58.9 (0.28)	60.8 (0.26)	62.8 (0.26)	64.3 (0.27)	<0.001
Sex, % women	28.3	45.3	61.4	79.3	<0.001
Race, n (%)					
Black	370 (26.8)	387 (26.5)	380 (26.1)	324 (23.1)	0.11
Chinese	131 (9.5)	164 (11.2)	204 (14.0)	188 (13.4)	<0.001
Hispanic	301 (21.8)	330 (22.6)	298 (20.5)	262 (18.7)	0.06
White	578 (41.9)	580 (39.7)	573 (39.4)	627 (44.8)	0.007
Education, n (%)					
Less than HS	233 (16.9)	247 (16.9)	246 (16.9)	212 (15.1)	0.46
HS graduate	237 (17.2)	251 (17.2)	262 (18.0)	269 (19.2)	0.53
Some college	381 (27.6)	395 (27.0)	447 (30.7)	403 (28.8)	0.16
College graduate	237 (17.2)	267 (18.2)	247 (17.0)	269 (19.2)	0.42
Graduate school	292 (21.1)	301 (20.6)	252 (17.4)	248 (17.7)	0.03
Current smoking, % (SE)	14.8 (0.01)	12.4 (0.01)	13.2 (0.01)	12.0 (0.01)	0.17
Current alcohol use, % (SE)	42.5 (0.01)	43.9 (0.01)	41.0 (0.01)	41.1 (0.01)	0.31
BMI, kg/m^2	27.0 (0.14)	27.9 (0.13)	28.4 (0.13)	28.6 (0.14)	<0.001
Waist circumference, cm	94.5 (0.38)	97.1 (0.35)	97.69 (0.35)	98.8 (0.37)	<0.001
hs-CRP, mg/L	3.08 (0.16)	3.79 (0.15)	3.77 (0.15)	4.01 (0.16)	<0.001
Glucose, mg/dL	88.0 (0.28)	89.2 (0.26)	89.8 (0.26)	91.4 (0.28)	<0.001
Insulin, mU/L	8.5 (0.15)	9.5 (0.14)	9.7 (0.14)	10.3 (0.15)	<0.001
n-3, % of total*	5.79 (0.06)	5.59 (0.05)	5.61 (0.05)	5.58 (0.06)	0.03
EPA, % of total	0.99 (0.02)	0.88 (0.02)	0.87 (0.02)	0.86 (0.02)	<0.001
DHA, % of total	3.86 (0.04)	3.79 (0.03)	3.80 (0.03)	3.80 (0.04)	0.56

Adjusted for age, sex, race, education, and field center. n-3 FAs = EPA + DHA. *Low n-3 FA category <75th percentile; high n-3 FA category ≥75th percentile. HS, high school.

Table 2—Mean (SE) and frequency (%) of baseline characteristics by plasma phospholipid n-3 FA category,* MESA, N = 5,697

Characteristic	Low n-3 category** (n = 4,399)	High n-3 category** (n = 1,304)	P value
n-3 FAs, mean (SE)*	3.9 (0.02)	7.5 (0.03)	<0.001
EPA, % total	0.64 (0.01)	1.79 (0.02)	<0.001
DHA, % total	3.25 (0.01)	5.71 (0.03)	<0.001
Age, years, mean (SE)	61.3 (0.2)	63.0 (0.3)	<0.001
Sex, n (% women)	2,313 (53)	744 (59)	0.005
Race, n (%)			
Black	1,056 (24)	405 (31)	<0.001
Chinese	352 (8)	352 (27)	<0.001
Hispanic	1,012 (23)	156 (12)	<0.001
White	1,979 (45)	391 (30)	<0.001
Education, n (%)			
Less than HS	763 (17)	174 (14)	0.002
HS graduate	818 (19)	204 (15)	0.003
Some college	1,292 (29)	333 (26)	0.04
College graduate	770 (18)	250 (18)	0.83
Graduate school	750 (17)	343 (27)	<0.001
Current smoking, n (%)	653 (15)	92 (8)	<0.001
Current alcohol use, n (%)	1,820 (43)	573 (38)	<0.001
BMI, kg/m ²	28.2 (0.1)	27.3 (0.1)	<0.001
Waist circumference, cm	97.8 (0.2)	94.9 (0.4)	<0.001
hs-CRP, mg/dL	3.8 (0.1)	3.1 (0.1)	<0.001
Glucose, mg/dL	89.7 (0.2)	89.5 (0.3)	0.59
Insulin, mU/L	9.7 (0.1)	8.9 (0.2)	<0.001

Adjusted for age, sex, race, education, and field center. *n-3 FAs = EPA + DHA. **Low n-3 FA category <75th percentile; high n-3 FA category ≥75th percentile. HS, high school.

2 diabetes incidence was also conducted. Compared with those with lower plasma n-3 FA levels (<75th percentile), individuals with higher plasma n-3 FAs (≥75th percentile) showed a marginal 17% lower risk of developing type 2 diabetes (HR 0.83 [95% CI 0.68, 1.01]).

CONCLUSIONS

In a MESA subcohort of 5,697 participants over an 11-year follow-up period, we confirm previous findings that higher circulating NEFA levels increase risk for type 2 diabetes incidence. We extend

these findings by demonstrating a modifying influence of plasma n-3 FA levels on this association. Notably, no association between NEFAs and incident type 2 diabetes was observed for individuals with high levels of n-3 FAs, whereas those with lower n-3 FA levels have an increasing risk of type 2 diabetes across successive quartiles of NEFAs.

Though its interaction with n-3 FAs was previously unrecognized, the association of NEFAs with metabolic dysfunction and/or type 2 diabetes has been examined in a number of previous

studies. A cross-sectional study of Pima Indians (5) revealed that high NEFA levels was associated with type 2 diabetes after controlling for potential confounding factors including waist/thigh ratio and insulin-mediated glucose uptake (relative risk 2.3 [CI 1.1, 4.7]). A subsequent longitudinal study in the Paris Prospective cohort reported that higher fasting plasma NEFA concentrations conveyed a 30% higher risk for deterioration of glucose tolerance over a 2-year period (6). A more recent nested case-control study in the Atherosclerosis Risk in Communities study showed a 63% greater risk of type 2 diabetes development among middle-aged adults who had NEFA levels in the top quartile compared with those in the bottom quartile over a 9-year follow-up period (4).

In contrast to the above, early results from the prospective Medical Research Council Ely study showed that NEFA levels were not elevated in those with type 2 diabetes and were not associated with future type 2 diabetes (9). However, these null findings may have stemmed from power limitations, as only 21 individuals developed diabetes during the initial 4.5-year study period. A subsequent study in the same cohort was conducted in 2012 and showed that elevated NEFAs were associated with an approximate threefold increase in risk of type 2 diabetes or impaired fasting glucose over a 5–8-year period (7). Coupled with the above evidence from the Atherosclerosis Risk in Communities and Paris Prospective studies, elevations in NEFAs are a marker of disease risk independent of other risk factors including obesity and inflammation. However, there is also compelling evidence to suggest that elevated NEFA levels directly contribute to type 2 diabetes pathogenesis.

Despite the relatively few prospective studies of NEFAs and type 2 diabetes, experimental studies in cell culture, animal models, and humans have shown that NEFAs activate pathways known to promote disease development. Specifically, it has been demonstrated that elevated NEFAs increase lipid mediators such as diacylglycerol and ceramide (33–36) and induce inflammation (13–15), all of which may disrupt peripheral insulin-mediated signaling (13–18,36). Further illustrating their deleterious effects, pharmacological intervention to reduce NEFA levels was found to

Table 3—HRs (95% CIs) for the main effect associations of incident type 2 diabetes across quartiles of serum NEFAs, MESA, N = 5,697

Quartile, n	Quartiles of NEFAs				P _{trend}
	1 (n = 1,380)	2 (n = 1,461)	3 (n = 1,455)	4 (n = 1,401)	
Range	0.13–0.39	0.40–0.52	0.53–0.67	0.68–2.11	
Cases, n	124	166	181	186	
Model 1, HR (95% CI)	1.0	1.35 (1.07, 1.71)	1.58 (1.24, 2.00)	1.86 (1.45, 2.38)	<0.001
Model 2, HR (95% CI)	1.0	1.23 (0.97, 1.56)	1.35 (1.06, 1.72)	1.56 (1.21, 2.00)	0.006

Model 1 adjusted for age, sex, race, education, field center, smoking, and alcohol use. Model 2 adjusted for model 1 plus waist circumference and hs-CRP.

Table 4—Plasma phospholipid n-3 FAs modify the association between incident type 2 diabetes and serum NEFAs, presented as HRs (95% CIs), MESA, N = 5,697

Quartile, n	Quartiles of NEFAs				<i>P</i> _{trend}
	1 (n = 1,380)	2 (n = 1,461)	3 (n = 1,455)	4 (n = 1,401)	
Range	0.13–0.39	0.40–0.52	0.53–0.67	0.68–2.11	
					<i>P</i> _{interaction} = 0.03
Low n-3 (<75th percentile)*					
Cases, n	92	127	147	157	
Model 1, HR (95% CI)	1.0	1.41 (1.07, 1.84)	1.77 (1.35, 2.31)	2.18 (1.65, 2.88)	<0.001
Model 2, HR (95% CI)	1.0	1.30 (1.00, 1.70)	1.51 (1.15, 1.98)	1.83 (1.39, 2.43)	<0.001
High n-3 (≥75th percentile)*					
Cases, n	32	39	34	29	
Model 1, HR (95% CI)	1.0	1.17 (0.72, 1.89)	1.01 (0.60, 1.70)	0.96 (0.55, 1.66)	0.85
Model 2, HR (95% CI)	1.0	1.00 (0.61, 1.63)	0.89 (0.53, 1.51)	0.80 (0.46, 1.40)	0.83

Model 1 adjusted for age, sex, race, education, field center, smoking, and alcohol use. Model 2 adjusted for model 1 plus waist circumference and hs-CRP. *Low n-3 FA category <75th percentile; high n-3 FA category ≥75th percentile.

suppress inflammatory signaling and concomitantly increase insulin sensitivity in human subjects with type 2 diabetes (14). Taken together, high levels of NEFAs likely promote IR through multiple pathways including the accumulation of intracellular lipids and induction of inflammation. Whether these phenomena occur in individuals with chronically elevated NEFAs continues to be researched, but a pathophysiological role of NEFAs in type 2 diabetes is supported by the present findings.

The significant modifying influence of n-3 FAs on the relation between NEFAs and incident type 2 diabetes is a novel observation, and any number of mechanisms may account for this result (22–24,37). First, n-3 FAs and their metabolites, including the protectins and resolvins, may serve as direct antagonists to IR at systemic and local levels. In the case of the former, the DHA metabolite resolvin D1 has been shown to reduce IR by inducing expression of adiponectin (24), a systemic hormone well-documented for promoting insulin sensitivity (37). n-3 FAs have further been reported to act directly on peripheral tissues to reduce IR via the GPR120 receptor (22). n-3-stimulated activation of the GPR120 receptor has been shown to enhance GLUT4 translocation in adipocytes, facilitating glucose transport. Apart from their putative direct effects on IR, n-3 FAs may also actively suppress local inflammation. More specifically, n-3 FAs have been shown to reduce: 1) inflammatory markers C-reactive protein, interleukin-6, and tumor necrosis factor- α and associated signaling cascades; 2) activation of inflammatory immune cells;

3) macrophage accumulation in tissue; and 4) Toll-like receptor recruitment and activation (22–24). Taken together, n-3 FAs and their metabolites have been shown to activate mediators known to promote insulin sensitivity and suppress inflammation, both of which may counter type 2 diabetes development and/or progression. Importantly, the efficacy of n-3 FAs appears partially dependent on the study population, and health benefits may not be apparent without an underlying pathology, such as IR or inflammatory phenotype.

The current study has clinical, dietary, and future study implications. From a clinical perspective, serum NEFAs are not regularly assessed, and a standardized method with recognized physiological and pathophysiological ranges has yet to be established. It also remains unknown whether the risk information derived from assessing NEFA levels warrants the cost in the general population (<\$1.00/sample in the Wako assay, approved for diagnostic use). By comparison, assessing plasma n-3 FAs is likely cost-ineffective for the general population; however, recommendations to increase dietary n-3 FAs for those with high NEFA levels may be warranted and require further research. With respect to future studies of n-3 FA and type 2 diabetes incidence, it is important to recognize that IR and type 2 diabetes are conditions that often take years to develop, and short-term n-3 FA interventions may not be sufficient to detect improvements in affected individuals. It must further be acknowledged that dietary n-3 intake moderately correlates with levels of bioavailable n-3 FA in plasma and cell membranes (38), and

food preparation methods have a significant impact on their incorporation (39); thus, increased levels of n-3 FA intake may not necessarily translate to equivalent levels in bioavailable n-3 FAs. Taken together, long-term trials of n-3 FAs with direct assessments of FA and NEFA levels as well as multiple secondary end point markers for IR, insulin sensitivity, and/or inflammation may be better suited for evaluating a protective effect(s) of n-3 FAs.

The current study has a number of strengths as well as limitations. It is the first prospective study with a large multiethnic cohort showing that n-3 FAs modify the association between NEFAs and risk of incident type 2 diabetes. This association remained significant despite adjusting for multiple confounding factors, including those for adiposity and inflammation that are likely in the pathway of interest. In terms of limitations, NEFA levels have been reported to be highly variable, even in fasting subjects (40). Such volatility of NEFA measures can neither be confirmed nor refuted as they were not measured across multiple time points. In addition, plasma phospholipid FA levels were used as a proxy of bioavailable n-3 FAs, but represent only one pool of total plasma n-3 levels.

NEFA measurement may represent a tool for assessing type 2 diabetes risk independent of other risk factors including adiposity and inflammation. Further studies are warranted to establish NEFA ranges that identify moderate- and high-risk reference ranges for type 2 diabetes. The significant modifying influence of n-3 FAs (i.e., higher levels of plasma n-3 FAs) suggests a protective influence from disease development, but long-term trials or

intervention studies are needed to conclusively demonstrate whether higher n-3 FAs abrogate the risk conferred by NEFAs in the development of type 2 diabetes.

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