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INDIVIDUAL NON-ESTERIFIED FATTY ACIDS AND INCIDENT ATRIAL FIBRILLATION LATE IN LIFE

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Abstract

Objective: Obesity and dysmetabolism are major risk factors for atrial fibrillation (AF). Expansion of fat depots is associated with increased circulating total non-esterified fatty acids (NEFAs), elevated levels of which are associated with incident AF. We undertook comprehensive serum measurement of individual NEFA to identify specific associations with new-onset AF late in life.

Methods: The present study focused on participants with available serum and free of AF selected from the Cardiovascular Health Study, a community-based longitudinal investigation of older US adults. Thirty-five individual NEFAs were measured by gas chromatography. Cox regression was used to evaluate the association of individual NEFAs with incident AF.

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Contributorship Statement

Planning for this research was conducted by Drs. Pellegrini, Lichtenstein, Ix, Siscovick, Heckbert, Tracy, Mukamal, Djousse, and Kizer.

The project was conducted by Drs. Pellegrini, Buzkova, Lichtenstein, Mattan, Mukamal, Djousse, and Kizer.

All authors were involved in reporting the work. Dr. Pellegrini serves as guarantor.

Ethics Statement

This study complied with the Declaration of Helsinki. All participants provided written informed consent. CHS was approved by the Institutional Review Boards of the Coordinating Center (University of Washington) and field centers (University of Pittsburgh, Johns Hopkins University, Wake Forest University, and UC Davis).

Results: The study sample included 1,872 participants (age 77.7±4.4). During median follow-up of 11.3 years, 715 cases of incident AF occurred. After concurrent adjustment of all NEFAs and full adjustment for potential confounders, higher serum concentration of nervonic acid (24:1n-9), a long-chain monounsaturated fatty acid, was associated with higher risk of AF (HR per SD: 1.18; 95% CI: 1.08–1.29; p<0.001). Conversely, higher serum concentration of gamma-linolenic acid (18:3n-6), a polyunsaturated n-6 fatty acid, was associated with lower risk of AF (HR per SD: 0.81; 95% CI: 0.71–0.94; p=0.004). None of the remaining NEFAs was significantly associated with AF.

Conclusions: Among older adults, serum levels of non-esterified nervonic acid were positively associated, while serum levels of non-esterified gamma-linolenic acid were inversely associated, with incident AF. If confirmed, these results could offer new strategies for AF prevention and early intervention in this segment of the population at highest risk.

Keywords

atrial fibrillation; fatty acids; epidemiology

Journal Subject Terms:

Atrial Fibrillation; Metabolism; Epidemiology

Introduction

The prevalence of atrial fibrillation (AF) is expected to more than double in the next 3 decades in parallel with aging of the global population.[1] The arrhythmia has been categorized as an epidemic, with major implications for the public health in view of its attendant risks of hospitalization, stroke and cognitive impairment, heart failure, and mortality. Soaring rates of obesity are likewise of growing concern worldwide. Among its other adverse effects, obesity is a major modifiable risk factor for AF, accounting for nearly 20% of AF cases.[1] There are multiple mechanisms by which obesity can promote AF, such as increased left atrial size and pressure, induction of inflammation, and development of other comorbid conditions that themselves foster AF, such as sleep apnea and hypertension.[2] In addition, there are direct effects of visceral and cardiac fat depots which secrete bioactive factors, including non-esterified fatty acids (NEFAs).[3]

NEFAs play an important role in normal metabolism, serving as the key oxidative fuel for much of the body, and particularly the myocardium. Total circulating NEFA levels have been associated with various outcomes, including incident AF[4] and glucose dysregulation.[5] The mechanisms by which derangements in NEFA metabolism contribute to these outcomes is a matter of active study. Elucidating the associations of total circulating NEFA with AF depends on studying the contributions of individual NEFAs in order to pinpoint specific determinants and isolate potential therapeutic targets.

Prospective studies of individual fatty acids and cardiovascular outcomes have largely focused on plasma phospholipids, esterified fatty acids that reflect membrane composition rather than the balance between organ-tissue uptake, synthesis and release. Previous

measurement of circulating phospholipids, or of both esterified and non-esterified fatty acids, showed individual long-chain n-3 polyunsaturated fatty acids (PUFA) to relate to lower risk of AF in some,[6,7] but not all,[8,9] studies. Conversely, plasma-phospholipid long-chain saturated fatty acids (SFA) were linked to higher risk of incident AF.[10]

Available data on individual NEFAs and AF come from a small lipidomic study that compared patients with persistent AF who presented for cardioversion with age-, sex- and body-mass index-matched controls. Circulating levels of 18 individual NEFAs were significantly lower in the AF group compared to the control group, yet within the AF group, NEFA levels did not relate to likelihood of AF recurrence.[11] To date, no study has examined the association of individual circulating NEFAs with development of AF in older adults, the highest-risk group. To advance our previous work on total NEFA,[4] we undertook comprehensive serum measurement of individual NEFAs in the Cardiovascular Health Study to identify specific associations with new-onset AF late in life.

Methods

Study Population

CHS is a prospective cohort of adults ≥65 years from 4 US locations: Sacramento County, CA; Washington County, MD; Forsyth County, NC; and Allegheny County, PA. Random Medicare eligibility lists stratified by age and sex were used to recruit individuals and age-eligible household members at each site, of whom 57% agreed to participate.[12] An initial recruitment wave enrolled 5,201 individuals in 1989–90 (original cohort). Using the same recruitment approach, a second wave enrolled 687 predominantly African-American individuals in 1992–93 from all regions except Washington County (supplemental cohort). Entry criteria stipulated that individuals not be wheelchair dependent or institutionalized, not be currently receiving treatment for cancer, be able to give consent, and be expected to reside in their respective region for the upcoming 3 years.

CHS examinations involved medical history, medication inventory, physical examination, phlebotomy, and non-invasive testing. For the NEFA ancillary study, participants with available serum and eligible for a 2-hour oral glucose tolerance test (not on anti-hyperglycemic therapy) were included.

NEFA Measurement

Fasting serum was collected at the 1996–1997 exam. Blood was maintained at room temperature for 1 hour to allow clotting to occur, prior to centrifugation at 1110 × g for 10 minutes at 4°C, and subsequent storage at −80°C. Samples were stored at the central laboratory at the University of Vermont, Burlington, VT; analyses were performed in the Cardiovascular Nutrition Laboratory at Tufts University, Boston, MA. Serum was quick-thawed at 374°C for analysis, and lipids were extracted after addition of an internal standard (heptadecanoic acid dissolved in hexane) as previously described.[13] The NEFA fraction was separated using solid-phase chromatography (aminopropyl columns), then dried under nitrogen, saponified, and methylated to form fatty acid methyl esters as detailed previously.[13] An Autosystem XL gas chromatogram (Perkin Elmer, Boston, MA) equipped

with a 100 m × 0.25 mm capillary column (HP INNOWax, Agilent Technologies, CA) was used to identify 35 fatty acid peaks, with comparison to authenticated standards (NuCheck Prep, MN). The inter-assay coefficients of variation were 0.5% to 4.3% for fatty acids present at concentrations >5 mol%, 1.8 to 7.1% for fatty acids at concentrations between 1–5 mol%, and 2.8 to 11.1% for fatty acids at concentrations <1 mol%.

AF Ascertainment

The outcome of interest was incident AF or atrial flutter (throughout both are here referred to as AF). Ascertainment was based on annual electrocardiograms from 1989–90 to 1998–99, as well as ICD-9 codes from inpatient or outpatient records throughout follow-up. Episodes associated with coronary bypass or valve replacement surgery during the same hospitalization were excluded. The follow-up period concluded December 2014.

Covariates

Covariates were mostly assessed at the same visit (1996–97) when specimens for NEFA measurement were collected. When not available at the latter visit, measures (height and lipid fractions) were carried over from the 1992–93 visit. Age, sex, race, smoking, and alcohol consumption were based on self-report. Heavy and moderate alcohol consumption were defined as ≥14 drinks/week and 7–13 drinks/week, respectively, for men; and ≥7 drinks/week and 1–6 drinks/week, respectively, for women. Weight, height, and blood pressure were determined using standardized methods. Physical activity level was based on type and intensity of activities reported by participants. Glomerular filtration rate was estimated from serum cystatin C. Other laboratory measures included serum albumin and lipid panels, which were measured at the Central Laboratory soon after collection. Diabetes was defined as fasting glucose ≥126 mg/dL, random glucose ≥200 mg/dL, or use of anti-hyperglycemic medication. Prevalent CHD was determined by participant report of physician diagnosis of angina, myocardial infarction, or coronary revascularization prior to enrollment. This was validated by evidence from the baseline examination and record reviews. At follow-up, incident CHD, similarly defined, was adjudicated by the CHS Events Committee. Prevalent stroke was based on patient report of physician diagnosis, and validated as for CHD. Prevalent HF was assessed using self-report, confirmed by use of HF medications, physician questionnaires, and medical record review. The proportion of missing data for all individual covariates was <5%.

Statistical Analysis

Pairwise correlations for individual NEFAs or sub-classes of NEFAs, expressed as absolute serum concentrations, were assessed with Pearson coefficients. Cox regression was used to estimate the association of individual NEFAs with incident AF after accounting for potential confounders. We first evaluated associations with AF for individual NEFAs separately in adjusted models. We next proceeded to enter all 35 NEFAs in one model in order to derive mutually adjusted risk estimates. This analysis was considered the primary analysis, evaluating the associations of individual NEFAs independent of each other, obviating the need for multiple testing. Restricted cubic splines were assessed for departures from linearity; finding none, we report associations per SD increment in NEFA levels.

We modeled NEFA associations using two levels of adjustment for potential confounders. Such confounders were selected based on prior associations or known biological mechanisms. The initial model (Model 1) adjusted for age, sex, race, and clinic. The fully adjusted model (Model 2) accounted additionally for height, weight, physical activity, systolic blood pressure, antihypertensive medication use, diabetes, smoking status, alcohol intake, serum albumin, LDL cholesterol, HDL cholesterol, triglycerides, prevalent CHD, prevalent stroke, prevalent heart failure, and eGFR. In a sensitivity analysis, we limited follow-up to the first 5 years. We also assessed for effect modification by sex. To assess the impact of missing data, we additionally ran Model 1 only in those with complete Model 2 covariate data.

All analyses were conducted with R (R Development Core Team; http://www.r-project.org). Two-sided $P<0.05$ was considered statistically significant.

Patient and Public Involvement

Patients were not involved when the Cardiovascular Heart Study was designed in the late 1980s.

Results

Of the 4,413 individuals who took part in the 1996–97 examination, 2,139 had serum NEFA measurements. After exclusion of 267 participants with prevalent AF, 1,872 individuals were eligible for the present analyses (Figure S1). Characteristics of the study sample are presented in Table 1. Participants were on average late into their eighth decade, almost two-thirds were women, and about 1 in 7 was African-American. More than half had hypertension, but, reflecting the study design, a small minority had diabetes. Fewer than 1 in 5 had prevalent CHD, and small fractions had prevalent stroke or heart failure. As compared with excluded participants, the study sample was generally healthier, having a lower burden of cardiovascular risk factors, particularly diabetes, and less prevalent cardiovascular disease (data not shown).

Mean total saturated fatty acids accounted for 40% of the total serum NEFA concentration, with non-esterified palmitic acid and stearic acid making up most of this group (Figure 1 and Table 2). Monounsaturated fatty acids constituted another 37% of total serum NEFA, for which non-esterified oleic acid was the primary component; non-esterified nervonic acid was present in small amounts. Long-chain n-6 polyunsaturated fatty acids (n-6 PUFA) contributed 18%, most of which was non-esterified linoleic acid; non-esterified gamma-linolenic acid (GLA) was present in small amounts. Small amounts of *trans* fatty acids (3%) and n-3 PUFA (2%) made up the rest.

Figure 2 shows the pairwise correlations for individual NEFAs. These tended to be strong for medium-chain to long-chain saturated fatty acids with one another; and for medium-chain to long-chain monounsaturated fatty acids with one another, or with their saturated or *trans* fatty acid counterparts, but less so with their n-6 and n-3 polyunsaturated counterparts. Strong correlations were also observed for some n-6 or n-3 PUFAs with one another, as well as with *trans* fatty acids, which also exhibited strong correlations with one another.

During median follow-up of 11.3 years, 715 cases of incident AF occurred, an incidence rate of 4% per year. After full adjustment, several individual NEFAs exhibited significant relationships with incident AF, as shown in Table 2. For each SD increment in individual NEFA concentrations, these ranged from 5% lower relative hazards for arachidic acid, to 12% and 13% higher relative hazards for palmitoleic and nervonic acid.

When all 35 individual NEFAs were entered in the Cox models concurrently, two individual NEFAs showed significant associations with incident AF in both minimally and fully adjusted models, as shown in Table 3. In these analyses, assessment for inflation of the standard errors did not reveal meaningful problems with collinearity. After full adjustment, each SD increment in non-esterified nervonic acid, a long-chain monounsaturated fatty acid, exhibited a significant 18% higher hazard for AF. Conversely, every SD increment of non-esterified GLA, an n-6 fatty acid, was associated with a significant 19% lower hazard for AF. Findings were similar when the follow-up period was restricted to the first 5 years. Stratified analyses did not reveal obvious effect modification by sex. Analysis excluding participants with any missing data (7.6%) did not meaningfully alter results.

Discussion

In this study of older adults with a high incidence of AF, we found two individual NEFAs, nervonic acid (24:1n-9) and GLA (18:3n-6), to be strongly and significantly associated with this outcome after concurrent adjustment of all NEFAs and full adjustment for potential confounders. Higher serum concentration of non-esterified nervonic acid was associated with a higher risk of AF, while higher serum concentration of non-esterified GLA was associated with a lower risk of AF.

Nervonic acid, a long-chain monounsaturated fatty acid, occurs in the seed oils of rapeseed and mustard. It is consumed in low amounts by humans.[14] Nervonic acid can be synthesized de novo from stearic acid via desaturation to oleic acid, followed by three consecutive elongation reactions. To date, genome-wide association analyses in human populations have not identified variants associated with circulating nervonic acid levels.[15]Best known for its role in the nervous system, nervonic acid is an intermediate in the biosynthesis of myelin and is one of the major fatty acids in brain sphingolipids. Nervonic acid has also been associated with increased markers of inflammation and endothelial activation, as well as higher incidence of heart failure and all-cause mortality.[16] Nervonic acid has not been examined in previous studies of blood or adipose tissue fatty acids, making this its first reported association with AF to our knowledge.

A dietary study among women showed that low monounsaturated fatty acid intake was associated with higher incidence of persistent, but not paroxysmal, AF.[17] Similarly, in the large randomized PREDIMED trial, a Mediterranean diet enriched with extra virgin olive oil, a plant oil rich in the monounsaturated fatty acid oleic acid, was protective against incident AF as compared to a control Mediterranean diet not enriched with extra virgin olive oil or enriched with nuts.[18] The nut-rich diet had a lower proportion of monounsaturated fat, indirectly suggesting the potential benefit of monounsaturated fatty acid intake. The

basis for the favorable impact on AF of a Mediterranean diet high in monounsaturated fats, specifically as relates to circulating nervonic acid levels, requires further study.

Conversely, oleic acid, a precursor of nervonic acid, has been shown to increase action potential duration and triggered activity in exposed mouse myocytes.[19] Calcium transients were increased and sodium handling was likewise dysregulated. These findings support a possible mechanism whereby monounsaturated fatty acids could directly potentiate atrial arrhythmias.

GLA is an n-6 fatty acid present in various botanical seed oils and dietary supplements.[20] GLA is the metabolic product of desaturation of linoleic acid by delta-6 desaturase. It is lengthened to dihomo-gamma-linolenic acid (DGLA) by elongase 5 and converted to arachidonic acid (AA) by delta-5 desaturase.[20] Both DGLA and AA are substrates for cyclooxygenases and lipoxygenases. For AA, these enzymes lead to generation of pro-inflammatory eicosanoids, namely, 2-series prostaglandins and 4-series leukotrienes.[20] In the case of DGLA, however, the resulting species have anti-inflammatory properties.[20] These include prostaglandin E_1, which affects immune system function and at low levels has been shown to be antiarrhythmic,[21] and thromboxane A_1, which has vasodilatory and platelet-inhibiting effects.[22]

To our knowledge, the finding of an inverse association between circulating non-esterified GLA and new-onset AF is new. Studies of n-6 PUFA have been mixed with respect to impact on arrhythmias and other cardiac events. A Danish cohort study that examined dietary intake of PUFAs found no significant association with AF incidence.[23] A recent systematic review concluded that the effect of broadly increasing dietary PUFA was unclear with respect to AF, as evidence was of very low quality.[24]

As relates to PUFA levels in biospecimens, there was a suggestion of less AF following coronary bypass surgery among those with higher plasma AA levels.[25] Furthermore, low circulating DGLA levels and a low DGLA/AA ratio were associated with long-term mortality in patients with acute cardiovascular disease and heart failure.[26] Among a general cohort undergoing gluteal fat biopsy, wherein treelet analysis was used to derive clusters of adipocyte esterified and non-esterified fatty acids associated with incident AF, the n-6 PUFA cluster was found to be significantly inversely associated with the arrhythmia.[27] Notably, DGLA and AA, but not GLA, were part of the n-6 PUFA cluster, suggesting that GLA bears a weaker relationship with others in its sub-class, at least in gluteal fat.

A genome wide association study (GWAS) in the Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) consortium, which includes CHS, found a variant fatty acid desaturase cluster, rs174547,[28] which has been shown to have inverse associations with non-arrhythmic cardiovascular outcomes.[29] The rs174547 minor allele is associated with decreased delta-5, but increased delta-6, desaturase gene expression.[30] Interestingly, in the CHARGE GWAS, the minor allele was associated with higher DGLA and lower AA blood levels, as well as lower blood GLA and linoleic acid levels.[28] Thus, the findings with respect to non-arrhythmic cardiovascular disease would appear to run counter to the relationship between higher non-esterified GLA and lower AF risk documented here.

The association in our study only emerged, however, after simultaneous adjustment for circulating linoleic acid, DGLA and AA, as well as the remaining measured NEFAs. This suggests that the association observed with AF could relate to beneficial influences of low GLA levels with respect to this outcome that are otherwise masked by DGLA, AA, and other individual NEFAs. To our knowledge, no direct protective actions of GLA on immune, vascular or platelet function have been described, calling into question attribution of the association to direct GLA effects. But the biology of PUFAs is complex, especially with respect to the implications of circulating levels for membrane function and signaling in different tissue and cell compartments.[20] Additional study is necessary to determine the basis for the observed association, and whether it might be responsive to dietary or pharmacologic manipulation.

There are several limitations of our study. As a cohort study, the current analysis may illustrate associations, but cannot demonstrate causality. Individuals originally enrolled in this study represented the subset from random, stratified Medicare eligibility lists who gave consent. Moreover, participants had to survive and attend a follow-up examination up to 8 years later. By design, participants were largely free of diabetes, and overall had a lower burden of cardiovascular disease. Thus, the present results are not necessarily generalizable to the broader population of older adults. AF ascertainment was via annual ECG and inpatient and outpatient ICD-9 codes and therefore likely underrepresented true AF burden. Yet, as this was not expected to be different among the groups, any bias created should be toward the null.

In conclusion, the present study newly identifies positive and inverse associations of non-esterified nervonic acid and GLA with incident AF in older adults, the population at highest risk. These findings require replication in other cohorts. If confirmed, they suggest that enhanced understanding of mechanisms and approaches to modifying levels of these two NEFAs could offer new avenues to reducing the onset of this highly prevalent arrhythmia in our aging population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Questions

1. What is already known about this subject?

Prospective studies of individual fatty acids have largely focused on esterified fatty acids that reflect membrane composition rather than the balance between organ-tissue uptake, synthesis, or release. Long-chain n-3 polyunsaturated fatty acids (PUFA) have inconsistently related to lower risk of AF, while long-chain saturated fatty acids (SFA) have been linked to higher risk of incident AF.

2. What does this study add?

This study of older adults is the first to examine the association of individual circulating NEFAs with development of AF, and to document significant independent associations for two specific NEFAs. Nervonic acid was associated with a nearly 20% higher risk of AF per SD increment, and gamma-linolenic acid with a nearly 20% lower risk per SD increment.

3. How might this impact on clinical practice?

Identification of these two individual NEFAs as independently related to incident AF suggests that modification of their levels, or targeting of their associated pathways, could offer potential therapeutic approaches for prevention of AF. These findings provide impetus for replication and further understanding of the nexus between these individual NEFAs and their metabolism and this cardiac dysrhythmia.

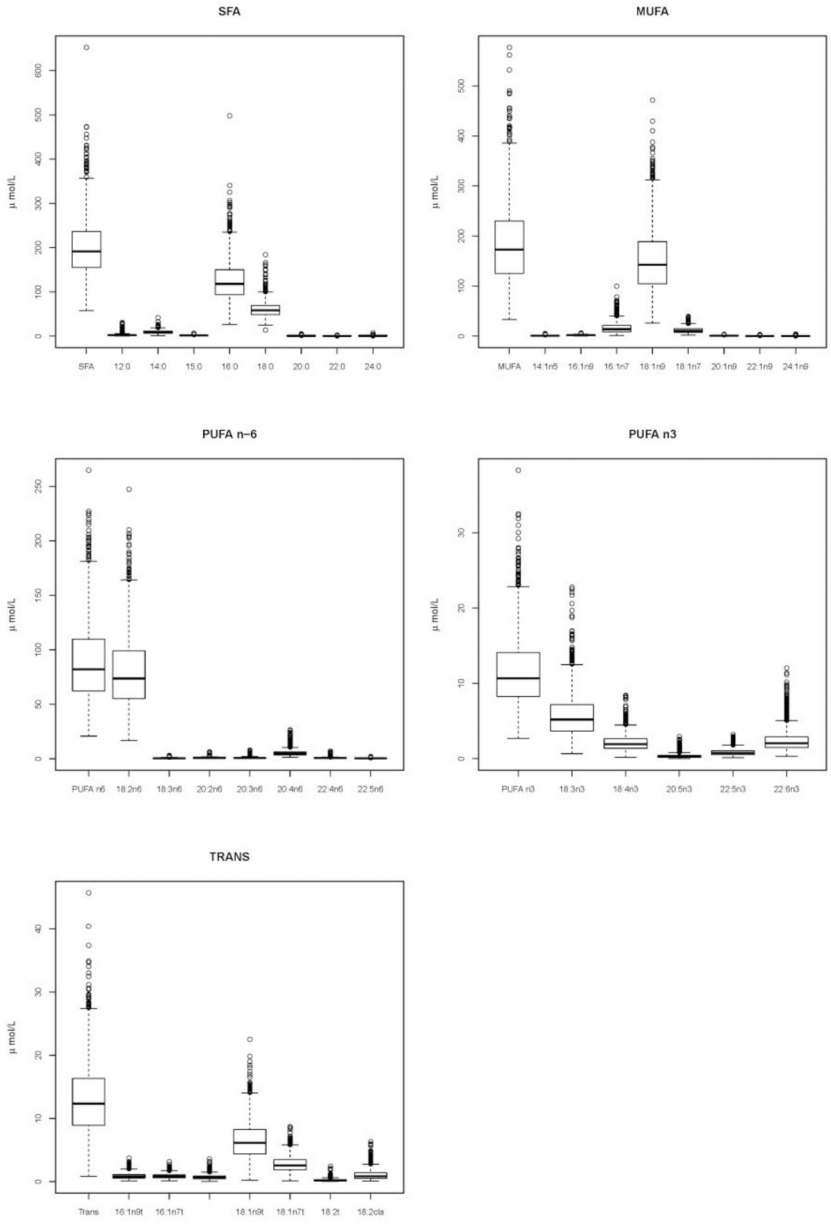

Figure 1.

Absolute values of serum non-esterified fatty acids. MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

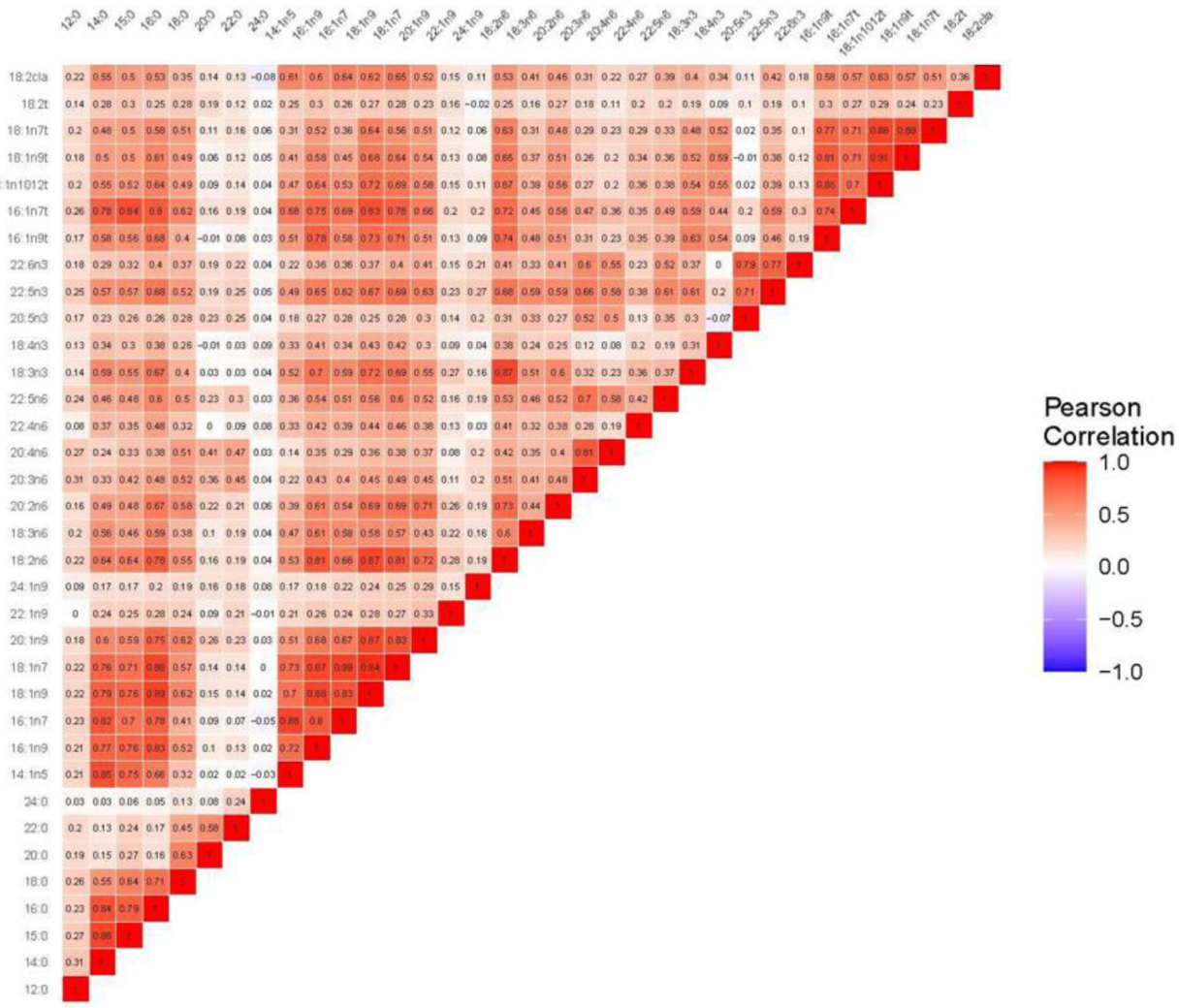

Figure 2.

Heat map visually depicting the correlation of the individual serum NEFAs with each other. The redder the box at their intersection, the more the NEFAs cluster together, as quantified by their positive Pearson correlation. Notably, many of the long-chain and medium-chain monounsaturated fatty acids are highly correlated with one another and with the saturated and trans fatty acids, but less so with the PUFAs. Strong correlations are also seen for some PUFAs with one another, and with trans fatty acids, which are also highly correlated with one another. NEFA, non-esterified fatty acid; PUFA, polyunsaturated fatty acid.

Table 1.

Participant characteristics*

Characteristics	Study Cohort (n=1872)
Age, yrs	77.7 ± 4.4
Women, n (%)	1165 (62)
Black, n (%)	266 (14)
Clinic, n (%)	
Forsyth County, NC	430 (23)
Sacramento County, CA	540 (29)
Washington County, MD	391 (21)
Allegheny County, PA	511 (27)
Height, m	1.63 ± 0.10
Weight, kg	71.6 ± 4.4
Systolic blood pressure, mmHg	137 ± 20
Diastolic blood pressure, mmHg	70 ± 11
Hypertension, n (%)	1124 (60)
Blood pressure medication use, n (%)	943 (51)
Diabetes, n (%)	49 (3)
Physical activity, n (%)	
None/little	408 (22)
Moderate	932 (50)
Strenuous	513 (28)
Smoking, n (%)	
Current user	149 (8)
Former user	782 (43)
Never user	911 (49)
Alcohol, n (%)	
Heavy use	153 (8)
Moderate use	677 (36)
LDL, mg/dL	130 ± 33
HDL, mg/dL	54 ± 14
Triglycerides, mg/dL	139 ± 77; 121 (89–166)
Serum albumin, g/dL	3.8 ± 0.3
eGFR, ml/min/1.73 m^2	73 ± 19
Prevalent coronary heart disease, n (%)	344 (18)
Prevalent heart failure, n (%)	79 (4)
Prevalent stroke, n (%)	86 (5)

*Mean ± standard deviation is presented for continuous variables and number (percent) for categorical variables; median (IQR) additionally provided for triglycerides, which showed substantial positive skew.

N = number, Yrs = years, m = meters, kg = kilograms, mmHg = milometers of mercury, mg/dL = milligrams per deciliter, g/dL = grams per deciliter, ml/min = milliliters per minute, m^2 = meters squared.

Table 2.

Serum concentrations and associations with incident atrial fibrillation for specific non-esterified fatty acids assessed in separate multivariable models.

NEFA		Serum Concetration (μmol/L)		Model 1		Model 2	
Trivial or Systematic Name	**Abbrev**	**Mean ± SD**	**Median (IQR)**	**HR per SD (95% CI)**	**P value**	**HR per SD (95% CI)**	**P value**
SATURATED FATTY ACIDS							
Lauric acid	12:0	2.65 ± 2.56	2.07 (1.38–3.07)	0.98 (0.91–1.06)	0.576	1.01 (0.93–1.08)	0.891
Myristic acid	14:0	9.00 ± 4.06	8.15 (6.14–11.19)	1.06 (0.97–1.15	0.198	**1.10 (1.00–1.20)**	**0.044**
Pentadecylic acid	15:0	1.61 ± 0.54	1.53 (1.24–1.90)	1.03 (0.95–1.11)	0.473	1.07 (0.98–1.17)	0.111
Palmitic acid	16:0	124.33 ± 44.50	117.96 (93.48–150.00)	1.06 (0.98–1.15)	0.180	1.09 (0.99–1.19)	0.075
Stearic acid	18:0	60.31 ± 17.22	58.16 (48.58–69.18)	0.99 (0.92–1.07)	0.858	1.01 (0.93–1.09)	0.824
Arachidic acid	20:0	0.73 + 0.38	0.62 (0.50–0.82)	0.94 (0.87–1.02)	0.133	0.95 (0.87–1.03)	0.208
Behenic acid	22:0	0.43 ± 0.18	0.39 (0.33–0.48)	0.95 (0.88 – 1.032	0.241	0.97 (0.89–1.05)	0.469
Lignoceric acid	24:0	0.65 ± 0.28	0.61 (0.50–0.74)	0.99 (0.91–1.08)	0.806	1.02 (0.94–1.11)	0.618
MONOUNSATURATED FATTY ACIDS							
Myristoleic acid	14:1n-5	0.87 ± 0.64	0.69 (0.43–1.15)	1.08 (1.00–1.17)	0.063	**1.11 (1.01–1.21)**	**0.026**
cis-7-hexadecenoic acid	16:1n-9	2.00 ± 0.86	1.85 (1.37–2.47)	1.07 (0.99–1.16)	0.086	**1.10 (1.01–1.20)**	**0.028**
Palmitoleic acid	16:1n-7	16.61 ± 11.18	13.84 (8.68–21.29)	**1.09 (1.01–1.19)**	**0.034**	**1.12 (1.02–1.22)**	**0.014**
Oleic acid	18:1n-9	150.92 ± 63.19	142.91 (104.65–188.86)	1.04 (0.96–1.13)	0.324	1.07 (0.98–1.17)	0.133
Cis-Vaccenic acid	18:1n-7	11.47 ± 5.51	10.44 (7.45–14.53)	1.08 (1.00–1.17)	0.051	**1.10 (1.01–1.21)**	**0.027**
Gondoic acid	20:1n-9	1.03 ± 0.47	0.94 (0.69–1.28)	1.00 (0.93–1.09)	0.941	1.02 (0.94–1.11)	0.634
Erucic acid	22:1n-9	0.38 ± 0.22	0.33 (0.24–0.44)	0.95 (0.88–1.04)	0.260	0.97 (0.89–1.05)	0.470
Nervonic acid	24:1n-9	0.35 ± 0.18	0.32 (0.27–0.38)	**1.17 (1.09–1.25)**	**<0.001**	**1.13 (1.04–1.22)**	**0.004**
N-6 POLYUNSATURATED FATTY ACIDS							
Linoleic acid	18:2n-6	79.37 ± 32.88	73.71 (55.30–98.92)	1.03 (0.95–1.11)	0.506	1.07 (0.98–1.17)	0.125
Gamma-Linolenic acid (GLA)	18:3n-6	0.56 ± 0.32	0.49 (0.34–0.72)	0.97 (0.90–1.05)	0.499	0.97 (0.89–1.05)	0.437
Dihomolinoleic acid	20:2n-6	0.90 ± 0.44	0.82 (0.61–1.10)	1.00 (0.93–1.08)	0.925	1.02 (0.94–1.10)	0.689
Dihomo-Gamma-Linolenic acid	20:3n-6	0.96 ± 0.69	0.79 (0.58–1.13)	0.99 (0.92–1.07)	0.878	1.00 (0.93–1.09)	0.934
Arachidonic acid	20:4n-6	5.39 ± 2.96	4.74 (3.55–6.34)	0.96 (0.89–1.03)	0.242	0.97 (0.89–1.05)	0.423
Adrenic acid	22:4n-6	0.71 ± 0.49	0.61 (0.42–0.86)	1.04 (0.97–1.11)	0.289	1.03 (0.96–1.12)	0.394
Docosapentaenoic acid	22:5n-6	0.38 ± 0.21	0.34 (0.25–0.46)	1.00 (0.92–1.07)	0.902	1.03 (0.96–1.12)	0.418

NEFA		Serum Concetration (μmol/L)		Model 1		Model 2	
Trivial or Systematic Name	**Abbrev**	**Mean ± SD**	**Median (IQR)**	**HR per SD (95% CI)**	**P value**	**HR per SD (95% CI)**	**P value**
N-3 POLYUNSATURATED FATTY ACIDS							
Alpha Linolenic acid (ALA)	18:3n-3	5.79 ± 2.93	5.21 (3.68–7.20)	1.03 (0.95–1.12)	0.445	1.08 (0.9–1.18)	0.068
Stearidonic acid (SDA)	18:4n-3	2.14 ± 1.05	1.93 (1.40–2.65)	1.03 (0.95–1.11)	0.516	1.04 (0.96–1.13)	0.311
Eicosapentaenoic acid (EPA)	20:5n-3	0.37 ± 0.29	0.29 (0.19–0.45)	0.97 (0.90–1.15)	0.441	1.01 (0.93–1.09)	0.884
Docosapentaenoic acid (DPA)	22:5n-3	0.85 ± 0.43	0.77 (0.54–1.05)	1.01 (0.94–1.09)	0.777	1.05 (0.97–1.15)	0.221
Docosahexaenoic acid (DHA)	22:6n-3	2.43 ± 1.48	2.05 (1.49–2.91)	0.98 (0.91–1.05)	0.518	1.01 (0.93–1.09)	0.806
TRANS FATTY ACIDS							
trans-7-hexadecenoic acid	16:1n-9	0.89 ± 0.48	0.81 (0.54–1.12)	1.07 (0.99–1.16)	0.096	1.09 (1.00–1.20)	0.051
Palmitelaidic acid	16:1n-7	0.87 ± 0.35	0.82 (0.61–1.06)	1.04 (0.96–1.13)	0.339	1.06 (0.97–1.16)	0.202
Petroselinic acid	18:1n-1012 *	0.71 ± 0.37	0.65 (0.46–0.88)	1.07 (0.99–1.15)	0.108	1.08 (0.99–1.17)	0.080
Elaidic acid	18:1n-9	6.57 ± 2.94	6.16 (4.39–8.27)	1.02 (0.94–1.10)	0.673	1.05 (0.97–1.14)	0.257
Trans-Vaccenic acid	18:1n-7	2.75 ± 1.21	2.58 (1.88–3.45)	1.00 (0.93–1.08)	0.965	1.03 (0.95–1.12)	0.484
Linelaidic acid	18:2	0.23 ± 0.19	0.18 (0.10–0.30)	1.00 (0.93–1.07)	0.976	1.01 (0.94–1.09)	0.802
Conjugated Linoleic acid	18:2cla	1.06 ± 0.76	0.86 (0.50–1.42)	1.01 (0.93–1.10)	0.776	1.01 (0.93–1.11)	0.766

Model 1 included age, gender, race, and clinic site. N = 1872

Model 2 included model 1 factors as well as height, weight, physical activity score, systolic blood pressure, antihypertensive medication, diabetes, smoking status, alcohol intake, serum albumin low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, prevalent coronary heart disease, prevalent stroke, prevalent heart failure, and eGFR. N = 1730

* sum of 18:1n-10t, 18:1n-11t and 18:1n-12t

NEFA = non-esterified fatty acids, SD = standard deviation, Abbrev = abbreviation, μ mol/L = micromoles per liter, HR = hazard ratio, SD = standard deviation, CI = confidence interval

Table 3.

Associations with incident atrial fibrillation for specific non-esterified fatty acids after entering all 35 non-esterified fatty acids concurrently in multivariable models.

NEFA		Model 1		Model 2	
Trivial or Systematic Name	**Abbrev**	**HR per SD (95% CI)**	**P value**	**HR per SD (95% CI)**	**P value**
SATURATED FATTY ACIDS					
Lauric acid	12:0	0.97 (0.88–1.06)	0.492	0.99 (0.90–1.08)	0.806
Myristic acid	14:0	1.10 (0.83–1.47)	0.517	1.12 (0.83–1.50)	0.475
Pentadecylic acid	15:0	0.96 (0.76–1.21)	0.725	1.04 (0.81–1.32)	0.783
Palmitic acid	16:0	0.97 (0.74–1.28)	0.830	0.94 (0.70–1.27)	0.693
Stearic acid	18:0	1.17 (0.95–1.43)	0.134	1.16 (0.93–1.43)	0.190
Arachidic acid	20:0	0.87 (0.74–1.01)	0.059	0.85 (0.72–1.00)	0.054
Behenic acid	22:0	0.99 (0.88–1.12)	0.917	1.02 (0.90–1.16)	0.793
Lignoceric acid	24:0	0.98 (0.89–1.08)	0.652	1.01 (0.92–1.11)	0.827
MONOUNSATURATED FATTY ACIDS					
Myristoleic acid	14:1n-5	0.96 (0.76–1.21)	0.703	0.92 (0.73–1.16)	0.483
cis-7-hexadecenoic acid	16:1n-9	1.11 (0.88–1.41)	0.379	1.10 (0.85–1.41)	0.479
Palmitoleic acid	16:1n-7	1.06 (0.79–1.42)	0.700	1.15 (0.84–1.57)	0.396
Oleic acid	18:1n-9	0.69 (0.44–1.08)	0.104	0.76 (0.46–1.26)	0.290
Cis-Vaccenic acid	18:1n-7	1.35 (0.96–1.90)	0.081	1.25 (0.87–1.80)	0.236
Gondoic acid	20:1n-9	0.91 (0.72–1.14)	0.408	0.88 (0.69–1.13)	0.325
Erucic acid	22:1n-9	0.92 (0.84–1.01)	0.094	0.94 (0.85–1.03)	0.185
Nervonic acid	24:1n-9	**1.22 (1.14–1.31)**	**<0.001**	**1.18 (1.08–1.29)**	**<0.001**
N-6 POLYUNSATURATED FATTY ACIDS					
Linoleic acid	18:2n-6	1.27 (0.95–1.71)	0.111	1.27 (0.92–1.76)	0.153
Gamma-Linolenic acid	18:3n-6	**0.88 (0.78–0.99)**	**0.040**	**0.81 (0.71–0.94)**	**0.004**
Dihomolinoleic acid	20:2n-6	0.91 (0.78–1.06)	0.225	0.89 (0.75–1.06)	0.193
Dihomo-Gamma-Linolenic acid	20:3n-6	1.10 (0.93–1.30)	0.282	1.04 (0.87–1.24)	0.679
Arachidonic acid	20:4n-6	0.91 (0.79–1.05)	0.196	0.91 (0.78–1.06)	0.231
Adrenic acid	22:4n-6	1.01 (0.93–1.11)	0.775	0.98 (0.89–1.08)	0.691
Docosapentaenoic acid	22:5n-6	0.95 (0.84–1.08)	0.424	1.02 (0.90–1.16)	0.712
N-3 POLYUNSATURATED FATTY ACIDS					
Alpha Linolenic acid (ALA)	18:3n-3	0.92 (0.76–1.11)	0.368	0.97 (0.80–1.19)	0.784
Stearidonic acid (SDA)	18:4n-3	1.00 (0.91–1.11)	0.977	1.00 (0.90–1.11)	0.979
Eicosapentaenoic acid (EPA)	20:5n-3	0.98 (0.84–1.13)	0.734	0.99 (0.85–1.15)	0.850
Docosapentaenoic acid (DPA)	22:5n-3	1.11 (0.90–1.36)	0.332	1.16 (0.94–1.44)	0.167
Docosahexaenoic acid (DHA)	22:6n-3	0.92 (0.79–1.08)	0.303	0.93 (0.79–1.09)	0.378
TRANS FATTY ACIDS					
trans-7-hexadecenoic acid	16:1n-9	0.92 (0.73–1.17)	0.500	0.92 (0.71–1.20)	0.535
Palmitelaidic acid	16:1n-7	1.03 (0.82–1.29)	0.801	0.92 (0.72–1.18)	0.523

NEFA		Model 1		Model 2	
Trivial or Systematic Name	**Abbrev**	**HR per SD (95% CI)**	**P value**	**HR per SD (95% CI)**	**P value**
Petroselinic acid	18:1n-1012*	1.44 (1.13–1.82)	0.003	1.28 (0.99–1.66)	0.058
Elaidic acid	18:1n-9	0.84 (0.67–1.06)	0.147	0.95 (0.75–1.22)	0.696
Trans-Vaccenic acid	18:1n-7	0.88 (0.71–1.10)	0.272	0.93 (0.73–1.18)	0.550
Linelaidic acid	18:2	1.00 (0.93–1.09)	0.929	1.01 (0.92–1.10)	0.907
Conjugated Linoleic acid	18:2cla	0.95 (0.84–1.07)	0.375	0.94 (0.83–1.07)	0.365

Model 1 included age, gender, race, and clinic site.

Model 2 included model 1 factors as well as height, weight, smoking status, alcohol usage, physical activity, blood pressure, LDL, HDL, albumin, eGFR, antihypertensive use, prevalent disease (diabetes, hypertension, coronary heart disease, heart failure, and stroke), and other individual NEFAs.

*sum of 18:1n-10t, 18:1n-11t and 18:1n-12t

NEFA = non-esterified fatty acids, SD = standard deviation, Abbrev = abbreviation, μ mol/L = micromoles per liter, HR = hazard ratio, SD = standard deviation, CI = confidence interval