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The determinants of fasting and post-load non-esterified fatty acids in older adults: The cardiovascular health study☆

Yakubu Bene-Alhasan [a,b], David S. Siscovick [c], Joachim H. Ix [d], Jorge R. Kizer [e], Russell Tracy [f], Luc Djoussé [g,**], Kenneth J. Mukamal [b,*]

[a] Department of Medicine, MedStar Union Memorial Hospital, Baltimore, MD, USA
[b] Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA
[c] The New York Academy of Medicine, New York, NY, USA
[d] Department of Medicine, University of California San Diego and Veterans Affairs San Diego Healthcare System, CA, USA
[e] Cardiology Section, San Francisco Veterans Affairs Health Care System, Departments of Medicine, Epidemiology and Biostatistics, University of California San Francisco, San Francisco, CA
[f] Department of Pathology and Laboratory Medicine, Larner College of Medicine, University of Vermont, Colchester, VT, USA
[g] Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, MA, USA

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ABSTRACT

Aim: Non-esterified fatty acids (NEFA) are potential targets for prevention of key cardiometabolic diseases of aging, but their population-level correlates remain uncertain. We sought to identify modifiable factors associated with fasting and post-load NEFA levels in older adults.
Methods: We used linear regression to determine the cross-sectional associations of demographic, anthropometric, and lifestyle characteristics and medication use with serum fasting and post-load NEFA concentrations amongst community-dwelling older adults enrolled in the Cardiovascular Health Study (n = 1924).
Results: Fasting NEFA levels generally demonstrated a broader set of determinants, while post-load NEFA were more consistently associated with metabolic factors. Waist circumference and weight were associated with higher fasting and post-load NEFA. Cigarette smoking and caffeine intake were associated with lower levels of both species, and moderate alcohol intake was associated with higher fasting levels whereas greater consumption was associated with lower post-load levels. Unique factors associated with higher fasting NEFA included female sex, higher age, loop and thiazide diuretic use and calcium intake, while factors associated with lower fasting levels included higher educational attainment, beta-blocker use, and protein intake. Hours spent sleeping during the daytime were associated with higher post-load NEFA, while DASH score was associated with lower levels.
Conclusion: Fasting and post-load NEFA have both common and unique modifiable risk factors, including sociodemographics, anthropometric, medications, and diet. Post-load NEFA were particularly sensitive to metabolic factors, while a broader range of determinants were associated with fasting levels. These factors warrant study as targets for lowering levels of NEFA in older adults.

1. Introduction

Numerous studies have established the clinical significance of circulating non-esterified fatty acids (NEFA) levels. Higher levels of fasting NEFA have been associated with increased risk of insulin resistance [1], diabetes [2,3], dementia [4], atrial fibrillation [5], heart

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* Corresponding author. 1309 Beacon Street, 2nd Floor, Brookline, MA, 02446, USA.
** Corresponding author.
E-mail address: kmukamal@bidmc.harvard.edu (K.J. Mukamal).

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failure [6], and hospitalization [7] amongst others. Although their associations with other outcomes are less certain, post-load NEFA levels appear to be strong correlates of insulin resistance [8]. Given the role of NEFA in adverse outcomes associated with aging, a better understanding of their determinants in the population is imperative to guide future studies and inform public health interventions.

NEFA are normally present in circulation in modest concentrations with rapid turnover [9] and bound to albumin. The main source of circulating NEFA is the breakdown of stored triglycerides in adipose tissues mediated in great part by hormone-sensitive lipase. Normally under the inhibition of insulin, hormone-sensitive lipase acts in the fasting state when insulin levels are low to release NEFA as a breakdown product of lipolysis [10]. Other hormones that antagonize insulin such as cortisol [11] and epinephrine [12,13] also lead to elevated levels of circulating fatty acids in experimental models. To our knowledge, however, no studies have assessed the potentially modifiable determinants of fasting and post-load NEFA at the population level in older adults.

To address the associations of modifiable factors with NEFA levels, we assessed sociodemographics, diet, alcohol intake, smoking history, physical activity level, anthropometry, and commonly used medications in a highly-phenotyped cohort of older adults.

2. Methods

2.1. Study population

We studied participants from the Cardiovascular Health Study (CHS), a longitudinal study of community-dwelling older adults in four US communities that began in 1989–1990 [14]. Participants were recruited using a two-stage random sampling method. The cohort was created from Medicare eligibility lists with age and sex strata representative of older adults in the US. Eligibility criteria included all individuals living in the household of those chosen, who were 65 years or older at the baseline examination, able to give personal informed consent, and expected to remain within the community for the next 3 years. Those excluded from the study included people receiving hospice care, undergoing treatment for cancer, or wheelchair-bound individuals. An initial cohort of 5201 individuals was supplemented in 1992–1993 with 687 predominantly African-American individuals from three of the original four sites. Detailed information on the CHS recruitment, design, and rationale have been published [15,16]. Participants returned for annual field center visits through 1998–1999 and have been followed with biennial telephone calls since.

For these analyses, we used data from the 1996–1997 field center visit, at which a 75-g oral glucose tolerance test was performed and serum samples collected for long-term storage. A total of 2144 participants had blood samples taken for the OGTT. We excluded 223 participants with missing covariates. Hence, a final sample size of 1921 participants was used for these analyses.

2.2. Covariates

2.2.1. Demography

We included age, sex, race, clinic site, educational attainment, marital status, and total combined household income. Age and educational attainment were categorized because of possible non-linear associations.

2.2.2. Anthropometry

Technicians measured weight and waist circumference at the field center visits. To avoid collinearity, we *a priori* focused on waist circumference (WC) over weight because it is a stronger marker for insulin resistance in older adults [17] and since post-load NEFA levels are associated with insulin sensitivity [18]. Nonetheless, we conducted sensitivity analyses that tested weight and height together rather than waist circumference. We created sex-adjusted quartiles for WC, weight, and height to account for potential non-linear relationships.

2.2.3. Physical activity

Participants reported the number of blocks walked in the past week, the number of daytime hours spent sleeping and the number of hours spent in the night sleeping in 1996-7. We categorized the number of blocks walked and hours spent sleeping at night as quartiles.

2.2.4. Diet

Participants completed a semiquantitative food frequency questionnaire (FFQ) at the 1995–1996 clinic visit. Based on the FFQ, we created energy-adjusted macronutrients as previously described [19,20]. We also included variables for dietary intakes of sodium, calcium, and caffeine from the FFQ. To further account for dietary patterns, we conducted sensitivity analyses using a score that assessed adherence to the Dietary Approaches to Stop Hypertension (DASH) dietary pattern.

2.2.5. Medication and substance use

Participants reported prescription medication use with a validated medication inventory [21] at each visit. We *a priori* included commonly-used medications plausibly related to NEFA levels, including statins, beta-blockers, calcium channel blockers, thiazides, and loop diuretics. Current smoking status and alcohol consumption were also ascertained in 1996–1997.

2.3. Outcome

Fasting and post-load NEFA concentration were the respective outcome variables in the two models. NEFA concentrations were measured in fasting and post glucose load blood samples taken during the 1996/1997 visit and kept at −70 °C until they were measured by the Wako Enzymatic method as previously described [2].

We natural log-transformed concentrations of both fasting and post-load NEFA given their pronounced right skew.

2.4. Statistical analyses

We describe the background demographic characteristics of participants by fasting and post-load NEFA concentrations using medians (interquartile ranges). We report p-values for categories using the Kruskal-Wallis test. We conducted two multi-variable linear regression models, one with fasting NEFA as the outcome variable and the other with post-load NEFA as the outcome variable. We built these models by including demographic, anthropometric, dietary, and lifestyle factors, along with medication use, as independent variables. Where competing possibly collinear variables existed, we first included the most plausible variable but tested alternatives in secondary analyses. We calculated partial Spearman correlation coefficients (adjusted for demographic factors and serum albumin concentration) for categories where competing variables existed to compare our choices with the other variables available. Serum albumin concentration was added to all models since NEFA circulates in the bloodstream bound to albumin [22]. We examined the possibly non-linear relationship between continuous variables and fasting and post-load NEFA levels non-parametrically with restricted cubic splines [23] and categorized continuous variables into quantiles when found to be non-linear. Finally, we conducted a sensitivity analysis by adjusting for prevalent cardiovascular disease (myocardial infarction or stroke) and kidney disease (eGFR <30). No formal correction for multiple testing was done on account of strong prior hypotheses used in building the models, hence p-values should be interpreted with caution. Analyses were carried out in SAS (SAS Institute, Cary, North Carolina) and R (R Core Team, 2014).

3. Results

Overall mean NEFA values were, as expected, substantially higher in the fasting state than post-load (0.34mEq/L vs 0.05mEq/L; $p < 0.001$). The sociodemographic-adjusted Spearman correlation coefficient between the two was 0.22 ($p < 0.001$).

3.1. Demography

Fasting NEFA levels were associated before adjustment with more demographic factors than were post-load NEFA levels. Median fasting NEFA concentrations varied significantly across strata of age, sex, educational attainment, marital status, and household income whereas median post-load NEFA concentration only varied significantly between strata of household income (Table 1). As expected, median fasting NEFA concentration was higher among women, but post-load NEFA concentration was similar among women and men (Fig. 1).

The findings were generally similar in fully adjusted models (Table 3t). After conditioning on all covariates, men had 26% ($p < 0.001$) lower mean fasting NEFA than females. Fasting NEFA concentration increased with increasing age and decreased with increasing level of educational attainment, both monotonically. In contrast, no demographic factor strongly influenced post-load NEFA values in fully adjusted analyses.

3.2. Anthropometry

WC was correlated with both fasting and post-load NEFA (Table 2); the correlations were nearly identical for BMI and were stronger for post-load than fasting NEFA. Likewise, we observed a monotonic relationship of WC with post-load but not fasting NEFA (Table 3). In general, the associations with measures of obesity were stronger than for any other covariate, particularly for post-load NEFA.

3.3. Sleep duration and physical activity

Every hour more of daytime sleep was associated with 5% ($p = 0.007$) higher post-load NEFA level. Spline analyses revealed a non-linear association between the numbers of hours spent sleeping at night and fasting NEFA, with a nadir at 7–8 h of night sleep (Fig. 2). Exercise intensity in Kcal/day and the number of blocks walked in the last week were weakly correlated with fasting NEFA (partial spearman r = -0.055 ($p = 0.02$) vs −0.058 ($p = 0.01$), respectively) and neither was significantly correlated with post-load NEFA ($p = 0.14$–0.28).

Table 1
Crude Fasting and Post-Load NEFA by demographic factors.

	Fasting NEFA (mEq/L) Median [IQR]	p-value	Post-load NEFA (mEq/L) Median [IQR]	p-value
Age (years)		<0.01		0.16
65–74	0.33 [0.24, 0.45]		0.05 [0.04, 0.08]	
75–84	0.35 [0.25, 0.46]		0.05 [0.04, 0.07]	
≥80	0.38 [0.28, 0.50]		0.05 [0.04, 0.07]	
Race		0.63		0.24
White	0.35 [0.26, 0.47]		0.05 [0.04, 0.07]	
Others	0.34 [0.25, 0.46]		0.05 [0.04, 0.07]	
Educational attainment		<0.01		0.17
Less than college education	0.36 [0.27, 0.48]		0.05 [0.04, 0.07]	
College education	0.38 [0.26, 0.47]		0.05 [0.04, 0.07]	
More than college education	0.32 [0.24, 0.44]		0.05 [0.03, 0.07]	
Sex		<0.01		0.06
Male	0.28 [0.21, 0.37]		0.05 [0.04, 0.08]	
Female	0.40 [0.29, 0.52]		0.05 [0.04, 0.07]	
Combined household income		<0.01		<0.01
<$16,000	0.37 [0.27, 0.50]		0.05 [0.04, 0.08]	
$16,000 – $34,999	0.34 [0.25, 0.46]		0.05 [0.04, 0.07]	
≥ $35,000	0.32 [0.23, 0.44]		0.05 [0.03, 0.07]	
Marital status		<0.01		0.72
Married	0.33 [0.24, 0.44]		0.05 [0.04, 0.07]	
Others	0.39 [0.28, 0.51]		0.05 [0.04, 0.07]	

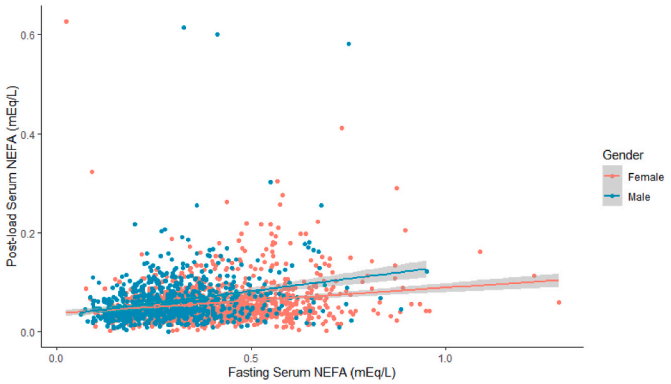

Fig. 1. Correlation between fasting and post-load NEFA by sex.

Table 2
Partially adjusted Spearman's Rank Coefficients for anthropometric, dietary and activity predictors.

	Fasting NEFA		Post-load NEFA	
	r (Spearman's)	p-value	r (Spearman's)	p-value
Anthropometry				
Waist Circumference	0.073	<0.01	0.182	<0.01
Weight	0.034	0.15	0.149	<0.01
4-year Change in weight	−0.029	0.21	0.059	0.013
Height	−0.075	<0.01	−0.002	0.93
BMI	0.072	<0.01	0.182	<0.01
Activity				
Blocks walked	−0.058	0.014	−0.026	0.28
Kcal/day	−0.055	0.02	−0.035	0.14
Daytime hours sleeping	0.074	0.002	0.064	<0.01
Hours of nighttime sleep	−0.003	0.91	0.023	0.33

Adjusted for demographic factors and serum albumin concentration.

3.4. Diet

After adjusting for total caloric intake and all other variables, protein intake was associated with lower mean fasting NEFA, but other macronutrients were not similarly associated; its association with post-load NEFA was of similar magnitude but not statistically significant. Among micronutrients, caffeine intake was associated with lower levels of both fasting and post-load NEFA. Conversely, dietary calcium intake was associated with higher fasting NEFA concentrations.

A model that used DASH scores instead of individual nutrients had similar estimates for the other independent variable, and each unit increase in DASH score was associated with 1% lower post-load NEFA levels ($p = 0.007$) (Table 4).

3.5. Medication and substance use

Similar to other categories of determinants, several medications were associated with fasting NEFA levels, but none was significantly associated with post-load NEFA. Beta-blocker use was associated with lower, while loop and thiazide diuretic use was each associated with higher fasting NEFA concentrations.

Smoking status was associated with lower fasting and post-load

Table 3
Fully adjusted model using energy-adjusted nutrients.

	Fasting NEFA		Post-load NEFA	
	% Change [95% CI]	p value	% Change [95% CI]	p value
Age (years)		0.003		0.36
65–74	REF		REF	
75–84	1.05 [1.00,1.10]	0.04	0.95 [0.89,1.02]	0.17
≥85	1.14 [1.06,1.24]	0.001	0.98 [0.88,1.10]	0.76
Male	0.74 [0.71,0.77]	<0.001	1.06 [0.99,1.13]	0.09
Married	0.96 [0.91,1.00]	0.06	0.99 [0.92,1.06]	0.76
Total combined household income		0.97		0.14
<$16,000	REF		REF	
$16,000 – $34,999	1.01 [0.96,1.05]	0.82	1.02 [0.95,1.10]	0.50
≥ $35,000	1.00 [0.95,1.06]	0.95	0.95 [0.88,1.04]	0.27
White	0.95 [0.90,1.01]	0.13	0.98 [0.90,1.06]	0.58
Educational attainment		0.05		0.68
Less than college education	REF		REF	
College education	0.97 [0.90,1.03]	0.29	0.96 [0.87,1.06]	0.39
More than college education	0.95 [0.90,0.99]	0.02	1.00 [0.93,1.07]	0.93
Waist circumference		0.001		<0.001
Quartile 1	REF		REF	
Quartile 2	0.98 [0.93,1.03]	0.45	1.14 [1.06,1.23]	0.001
Quartile 3	1.00 [0.95,1.05]	0.97	1.23 [1.14,1.33]	<0.001
Quartile 4	1.09 [1.03,1.15]	0.002	1.27 [1.18,1.38]	<0.001
Daytime hours spent sleeping	1.02 [1.00,1.04]	0.09	1.05 [1.01,1.08]	0.007
Hours spent sleeping at night		0.07		0.42
Quartile 1	REF		REF	
Quartile 2	0.99 [0.94,1.04]	0.62	1.04 [0.97,1.12]	0.26
Quartile 3	0.96 [0.91,1.01]	0.09	1.03 [0.96,1.11]	0.36
Quartile 4	1.04 [0.97,1.11]	0.27	1.08 [0.98,1.19]	0.11
Number of blocks walked last week		0.28		0.93
Quartile 1	REF		REF	
Quartile 2	1.01 [0.95,1.06]	0.84	0.98 [0.91,1.06]	0.67
Quartile 3	1.00 [0.95,1.06]	0.88	0.99 [0.92,1.08]	0.89
Quartile 4	0.96 [0.91,1.01]	0.14	0.98 [0.90,1.06]	0.57
Calcium channel blocker use	1.01 [0.96,1.06]	0.67	1.03 [0.96,1.11]	0.41
Loop diuretic use	1.13 [1.05,1.21]	0.001	1.06 [0.96,1.18]	0.24
Thiazide diuretic use	1.09 [1.03,1.15]	0.001	1.03 [0.96,1.11]	0.39
Statin use	0.96 [0.90,1.02]	0.23	1.03 [0.94,1.13]	0.48
Beta blocker use	0.84 [0.80,0.89]	<0.001	1.02 [0.94,1.10]	0.61
Carbohydrate intake [1] (per 10g)	0.99 [0.98,1.01]	0.46	1.00 [0.98,1.02]	0.92
Protein intake [1] (per 10g)	0.97 [0.95,1.00]	0.02	0.97 [0.94,1.01]	0.10
Animal fat intake [1] (per 10g)	1.00 [0.96,1.04]	0.97	1.06 [1.00,1.12]	0.06
Vegetable fat intake [1] (per 10g)	0.98 [0.94,1.02]	0.25	0.99 [0.93,1.04]	0.61
Dietary fibre intake [1] (per 10g)	0.98 [0.96,1.02]	0.32	0.98 [0.94,1.03]	0.44
Calcium intake [2]	1.03 [1.01,1.06]	0.007	1.01 [0.98,1.04]	0.62
Sodium intake [2]	0.99 [0.97,1.01]	0.44	1.01 [0.98,1.05]	0.47
Caffeine intake [2]	0.98 [0.96,1.00]	0.02	0.97 [0.94,1.00]	0.048
Smoking status		0.047		0.005
Never	REF		REF	
Former	0.99 [0.95,1.03]	0.57	1.00 [0.94,1.06]	0.97
Current	0.91 [0.84,0.98]	0.01	0.83 [0.74,0.94]	0.002
Alcohol consumption (drinks/week)		0.008		0.003
None (for >4 years)	REF		REF	
None (for ≤4 years)	1.03 [0.97,1.10]	0.33	1.01 [0.92,1.12]	0.77
1–7	0.98 [0.94,1.03]	0.43	1.05 [0.99,1.13]	0.13
8–14	1.16 [1.05,1.29]	0.004	1.04 [0.89,1.20]	0.64
>14	1.06 [0.93,1.21]	0.36	0.77 [0.63,0.93]	0.007

1-Energy-adjusted macronutrients. 2-Per standard deviation change. Also adjusted for clinic site and serum albumin concentration.

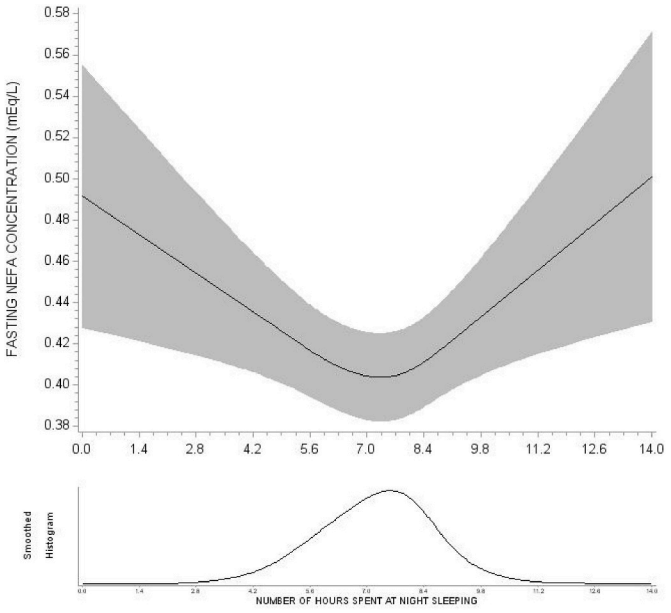

Fig. 2. Fully adjusted spline analysis of fasting NEFA concentration (mEq/L) by hours of sleep per night.
1. Test for curvature (i.e. non linear relation): p-value is: 0.0020.
2. Test for overall significance of the curve: p-value is: 0.0065.

NEFA concentrations, compared to participants who had never smoked, even in analyses adjusted for adiposity. Alcohol consumption was also significantly associated with fasting and post-load NEFA concentrations, but in complex ways. Participants who consumed 8 or more drinks had higher mean fasting NEFA levels than those who had no drinks for over 4 years. In contrast, those who consumed more than 14 drinks had a lower mean post-load NEFA concentration. Our results remained the same for

Table 4
Fully adjusted model using DASH scores.

	Fasting NEFA		Post-load NEFA	
	% Change [95% CI]	p value	% Change [95% CI]	p value
Age (years)		0.001		0.37
65–74	REF		REF	
75–84	1.05 [1.00,1.10]	0.03	0.96 [0.90,1.03]	0.23
≥85	1.15 [1.07,1.25]	<0.001	1.00 [0.90,1.13]	0.93
Male	0.73 [0.70,0.77]	<0.001	1.06 [0.99,1.13]	0.11
Married	0.96 [0.91,1.00]	0.07	0.99 [0.93,1.06]	0.85
Total combined household income		0.96		0.17
<$16,000	REF		REF	
$16,000 – $34,999	1.01 [0.96,1.06]	0.79	1.02 [0.95,1.09]	0.58
≥ $35,000	1.01 [0.95,1.07]	0.83	0.95 [0.88,1.04]	0.27
White	0.97 [0.91,1.02]	0.25	1.00 [0.92,1.09]	0.98
Educational attainment		0.039		0.64
Less than college education	REF		REF	
College education	0.97 [0.90,1.03]	0.29	0.96 [0.87,1.05]	0.35
More than college education	0.94 [0.90,0.99]	0.01	1.00 [0.93,1.06]	0.91
Waist circumference		<0.001		<0.001
Quartile 1	REF		REF	
Quartile 2	0.98 [0.93,1.04]	0.51	1.14 [1.06,1.23]	0.001
Quartile 3	1.00 [0.95,1.05]	0.99	1.24 [1.14,1.33]	<0.001
Quartile 4	1.09 [1.03,1.15]	0.002	1.28 [1.18,1.38]	<0.001
Daytime hours spent sleeping	1.02 [1.00,1.04]	0.08	1.05 [1.01,1.08]	0.005
Hours spent sleeping at night		0.07		0.40
Quartile 1	REF		REF	
Quartile 2	0.99 [0.94,1.04]	0.60	1.04 [0.97,1.12]	0.26
Quartile 3	0.96 [0.91,1.01]	0.08	1.04 [0.97,1.11]	0.31
Quartile 4	1.04 [0.97,1.10]	0.30	1.08 [0.98,1.19]	0.11
Number of blocks walked last week		0.21		0.95
Quartile 1	REF		REF	
Quartile 2	1.01 [0.96,1.06]	0.72	0.98 [0.91,1.06]	0.69
Quartile 3	1.00 [0.95,1.06]	0.86	0.99 [0.92,1.07]	0.87
Quartile 4	0.96 [0.90,1.01]	0.13	0.98 [0.90,1.06]	0.58
Calcium channel blocker use	1.01 [0.96,1.06]	0.78	1.03 [0.96,1.10]	0.48
Loop diuretic use	1.14 [1.06,1.22]	0.001	1.08 [0.97,1.19]	0.17
Thiazide diuretic use	1.09 [1.03,1.14]	0.002	1.03 [0.96,1.11]	0.40
Statin use	0.96 [0.90,1.02]	0.20	1.02 [0.93,1.12]	0.61
Beta blocker use	0.84 [0.80,0.89]	<0.001	1.02 [0.95,1.11]	0.54
Smoking status		0.03		0.003
Never	REF		REF	
Former	0.98 [0.94,1.02]	0.42	0.99 [0.94,1.06]	0.86
Current	0.90 [0.83,0.97]	0.007	0.83 [0.74,0.93]	0.001
Alcohol consumption (drinks/week)		<0.001		0.002
None (for >4 years)	REF		REF	
None (for ≤4 years)	1.03 [0.97,1.10]	0.32	1.02 [0.93,1.13]	0.63
1–7	0.99 [0.94,1.03]	0.56	1.06 [0.99,1.13]	0.11
8–14	1.19 [1.08,1.30]	<0.001	1.04 [0.92,1.19]	0.53
>14	1.12 [1.01,1.24]	0.03	0.78 [0.68,0.91]	0.001
DASH Score	1.00 [0.99,1.00]	0.63	0.99 [0.99,1.00]	0.007

Also adjusted for clinic site and serum albumin concentration.

all independent variables after adjusting for prevalent cardiovascular and kidney disease in sensitivity analyses.

4. Discussion

In this cohort of well-phenotyped community-dwelling older adults, fasting NEFA concentrations tended to relate to a wide variety of demographic, anthropometric, and lifestyle factors. Fasting NEFA levels were higher with female sex, increasing age, low educational attainment, WC, diuretic use as well as calcium intake and decreased with protein intake, caffeine intake and smoking. In contrast, WC or weight were major determinants of post-load NEFA concentration [were largely metabolic]. These differences highlight both the potentially modifiable nature of NEFAs and the particular specificity of post-load NEFA in relation to adiposity.

Our findings related to sex were noteworthy. We observed women to have higher mean fasting NEFA concentrations than men. In contrast, they had lower post-load NEFA, presumably reflecting greater insulin sensitivity and therefore greater drop in response to insulin secretion during the OGTT. We have also observed sexual dimorphism [24] in the associations of fasting and post-load NEFA with incident diabetes, where fasting NEFA are more strongly associated with risk among men but post-load predominate among women, suggesting that the observed differences at baseline have potentially important longer-term consequences.

4.1. Anthropometry

Previous studies have reported an association between increasing weight, WC, and hip circumference and increases in the concentration of NEFA [1,25,26]. Our study results corroborate the findings of these studies. We were not able to readily distinguish between overall and central obesity in their associations with fasting or post-load NEFA, as both BMI and WC were strongly associated with both endpoints. However, our results do point to the particular importance of metabolic factors like adiposity with post-load NEFA, as the associations with adiposity were considerably stronger than for fasting NEFA and represented one of the few significant determinants of post-load NEFA.

4.2. Physical activity and sleep duration

Physical exertion appears to have complex associations with NEFA, as it may lead to acute drops (presumably related to usage by muscle cells for fuel) followed by a steady rise afterward, with the elevation varying by the intensity of physical activity [27,28]. However, NEFA levels appear to return to baseline within days [29], and our fully adjusted models did not show any significant associations with measures

of physical activity after adjustment for weight.

We observed a novel association between daytime sleep and higher post-load NEFA. Of note, about 90% of CHS participants did not sleep during the day, and the association is likely to be confounded, at least in part, by the many factors that may lead to daytime sleepiness. Sleep apnea, for example, is clearly associated with insulin resistance [30]. In contrast, nighttime sleep was not associated with post-load NEFA but spline analyses suggested that intermediate levels of sleep (i.e., 7–8 h) are associated with lower fasting NEFA.

4.3. Diet

Our study showed that an isocaloric increase in protein intake was associated with lower mean fasting (and possibly post-load) NEFA, which concords with previous studies that have linked protein intake with improved insulin sensitivity [31–33]. Of note, we measured NEFA responses to a pure glucose load, and not a post-prandial response to mixed meals, which leads to a less predictable insulin response. Adherence to a DASH diet was associated with a modest decrease in post-load NEFA levels, which also fits with its observed improvement in insulin sensitivity when combined with other lifestyle changes [34].

Our findings with caffeine intake were somewhat counterintuitive. Caffeine has long been known to increase levels of circulating epinephrine [35,36] and cortisol [37]. We therefore hypothesized that caffeine intake would lead to increased levels of NEFA. Many previous trials have produced results consistent with this [38–40]. Our study found caffeine intake to be associated with decreased levels of both fasting and post-load NEFA, suggesting that other pathways are likely involved. Caffeine has been shown *in vitro* to increase proliferation and reduce the apoptosis of β cells caused by fatty acids [41]. It is therefore plausible that the immediate effect of caffeine is an increase in catecholamines and cortisol, while improving insulin sensitivity in the long term. Indeed, prospective studies have shown a lower incidence of diabetes amongst caffeine consumers [42,43].

4.4. Medication and substance use

Beta-blocker use was associated with a decrease in fasting NEFA whereas loop and thiazide diuretics were associated with increases. Beta-blockers block β-adrenergic receptors in the sympathetic system and this should lead to reduced lipolysis and production of NEFA. Altmaier et al. [44] also reported lower fasting NEFA in beta-blocker users. In contrast, diuretics have been associated with insulin resistance and risk of diabetes, which may account for their association with higher fasting NEFA [45]. However, diuretics are also used for several disease states characterized by heightened neurohormonal activation and we cannot exclude the possibility that some residual confounding also exists [46,47].

Smoking, like caffeine, has been reported to elevate catecholamines [48] and cortisol levels [49]. We therefore cannot readily explain its association with lower NEFA levels, even in weight-adjusted models. Likewise, alcohol consumption of 8–14 drinks per week was associated with higher fasting NEFA levels while even higher quantities were associated with lower post-load NEFA levels. This may reflect the complex mixture of effects of ethanol. Alcohol consumption has been linked to better insulin sensitivity [50], which may explain its association with lower post-load NEFA levels (which appear most tied to metabolic factors). At the same time, alcohol appears to interfere with hepatic peroxisome proliferator-activated receptor-α activity, which would then interfere with resting (i.e., fasting) NEFA metabolism by disrupting its feedback control of intracellular lipids [51].

4.5. Implications

Our study identified several potentially interesting associations between NEFA and modifiable lifestyle factors, which could be the subject of public health interventions. The findings of our study should guide future prospective, ideally interventional studies to establish causal associations. In addition, the differences in associations between fasting and post-load NEFA suggest these are biochemically distinct entities. Post-load NEFA is more strongly linked with predictors of insulin resistance; hinting that a higher post-load NEFA level could be a better predictor of endpoints linked to this cardinal metabolic abnormality.

4.6. Strength

To our knowledge, our study assessed for the first time the association of demographic, socioeconomic, lifestyle, anthropometric, and medication use with fasting and post-load NEFA in older adults. The plethora of variables included, as well as the granularity of details on these variables enabled us to not only produce a comprehensive assessment of the factors that could influence NEFA but also reduces the possibility of confounding and model misspecification. Finally, the large number of participants in our study also ensured we had sufficient power to detect associations.

4.7. Limitation

The cross-sectional design of our study precludes our ability to establish temporality between predictors and outcome, although it is unlikely that levels of NEFA *per se* modify lifestyle choices. Many of our variables relied on self-report, introducing the possibility of measurement error. We also relied upon a single measurement of fasting and post-load NEFA, which does not allow us to capture changes in NEFAs over time. Finally, as in any observational study, we recognize the possibility of residual confounding.

5. Conclusion

In this cohort of older adults, fasting and post-load NEFA appeared to be distinct entities reflecting some shared but many divergent determinants. Whereas fasting NEFA concentrations were associated with a miscellany of factors, ranging from sociodemographics, medication use, diet and anthropometric measures (WC and weight), post-load NEFA were associated distinctly with factors intimately associated with glucose and lipid metabolism. Nonetheless, our study provides clear evidence that both fasting and post-load NEFA levels are associated with modifiable risk factors. Once these factors have been corroborated in future studies, clinical and public health interventions could target these lifestyle factors with the aim of controlling NEFA levels and potentially reducing the risk of the many diseases of aging associated with higher NEFA concentrations.

CRediT authorship contribution statement

Yakubu Bene-Alhasan: Formal analysis, Visualization, Writing – original draft. **David S. Siscovick:** Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Joachim H. Ix:** Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Jorge R. Kizer:** Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Russell Tracy:** Investigation, Methodology, Project administration, Resources, Writing – review & editing. **Luc Djoussé:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – review & editing. **Kenneth J. Mukamal:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metop.2023.100261.

References

[1] Arner P, Rydén M. Fatty acids, obesity and insulin resistance. Obes Facts 2015;8:147–55.
[2] Djoussé L, et al. Plasma fatty acid-binding protein 4, nonesterified fatty acids, and incident diabetes in older adults. Diabetes Care 2012;35:1701–7.
[3] Huang X, et al. Interactive association of lipopolysaccharide and free fatty acid with the prevalence of type 2 diabetes: a community-based cross-sectional study. J. Diabetes Investig. 2019;10:1438–46.
[4] Mukamal KJ. Nonesterified fatty acids, cognitive decline, and dementia. Curr Opin Lipidol 2020;31:1–7.
[5] Khawaja O, et al. Plasma free fatty acids and risk of atrial fibrillation (from the Cardiovascular Health Study). Am J Cardiol 2012;110:212–6.
[6] Djousse L, et al. Serum individual nonesterified fatty acids and risk of heart failure in older adults. Cardiology 2021:1–8. https://doi.org/10.1159/000513917.
[7] Ahiawodzi PD, et al. Nonesterified fatty acids and hospitalizations among older adults: the cardiovascular health study. J. Gerontol. Ser. A 2020:glaa228. https://doi.org/10.1093/gerona/glaa228.
[8] Karakas SE, Almario RU, Kim K. Serum fatty acid binding protein 4, free fatty acids and metabolic risk markers. Metabolism 2009;58:1002–7.
[9] Kremmyda L-S, Tvrzicka E, Stankova B, Zak A. Fatty acids as BIOCOMPOUNDS: their role in human metabolism, health and disease - a review. Part 2: fatty acid physiological roles and applications in human health and disease. Biomed Pap 2011;155:195–218.
[10] Kraemer FB, Shen W-J. Hormone-sensitive lipase. J Lipid Res 2002;43:1585–94.
[11] Djurhuus CB, et al. Additive effects of cortisol and growth hormone on regional and systemic lipolysis in humans. Am J Physiol Endocrinol Metab 2004;286:E488–94.
[12] Millet L, Barbe P, Lafontan M, Berlan M, Galitzky J. Catecholamine effects on lipolysis and blood flow in human abdominal and femoral adipose tissue. J. Appl. Physiol. Bethesda Md 1985;85:181–8. 1998.
[13] Lafontan M, Berlan M. Fat cell adrenergic receptors and the control of white and brown fat cell function. J Lipid Res 1993;34:1057–91.
[14] Design, rationale and objectives | chs-nhlbi. https://chs-nhlbi.org/CHSDesc.
[15] Tell GS, et al. Recruitment of adults 65 years and older as participants in the cardiovascular health study. Ann Epidemiol 1993;3:358–66.
[16] Fried LP, et al. The cardiovascular health study: design and rationale. Ann Epidemiol 1991;1:263–76.
[17] Racette SB, Evans EM, Weiss EP, Hagberg JM, Holloszy JO. Abdominal adiposity is a stronger predictor of insulin resistance than fitness among 50–95 Year olds. Diabetes Care 2006;29:673–8.
[18] Carlson OD, et al. Contribution of non-esterified fatty acids to insulin resistance in the elderly with normal fasting but diabetic 2h post challenge plasma glucose levels: Baltimore longitudinal study of aging. Metabolism 2007;56:1444–51.
[19] Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27.
[20] Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr 1997;65:1220S–8S.
[21] Psaty BM, et al. Assessing the use of medications in the elderly: methods and initial experience in the cardiovascular health study. J Clin Epidemiol 1992;45:683–92.
[22] van der Vusse GJ. Albumin as fatty acid transporter. Drug Metabol Pharmacokinet 2009;24:300–7.
[23] Durrleman S, Simon R. Flexible regression models with cubic splines. Stat Med 1989;8:551–61.
[24] Shitole SG, et al. Fasting and postload nonesterified fatty acids and glucose dysregulation in older adults. Am J Epidemiol 2022;191:1235–47.
[25] Wang S, et al. Fasting serum free fatty acid composition, waist/hip ratio and insulin activity in essential hypertensive patients. Hypertens Res 2008;31:623–32.
[26] Liu J, et al. [Association between the metabolic syndrome and free fatty acid]. Zhonghua Xinxueguanbing Zazhi 2005;33:653–7.
[27] Gar C, et al. Altered metabolic and hormonal responses to moderate exercise in overweight/obesity. Metabolism 2020;107:154219.
[28] Antunes BM, Rossi FE, Oyama LM, Rosa-Neto JC, Lira FS. Exercise intensity and physical fitness modulate lipoproteins profile during acute aerobic exercise session. Sci Rep 2020;10:4160.
[29] Wilhelmsen A, et al. Chronic effects of high-intensity interval training on postprandial lipemia in healthy men. J Appl Physiol 2019;127:1763–71.
[30] Koh H-CE, et al. Effect of obstructive sleep apnea on glucose metabolism. Eur J Endocrinol 2022;186:457–67.
[31] Gunnerud UJ, Ostman EM, Björck IME. Effects of whey proteins on glycaemia and insulinaemia to an oral glucose load in healthy adults; a dose-response study. Eur J Clin Nutr 2013;67:749–53.
[32] van Loon LJ, Saris WH, Verhagen H, Wagenmakers AJ. Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. Am J Clin Nutr 2000;72:96–105.
[33] Sun L, Ranawana DV, Leow MK-S, Henry CJ. Effect of chicken, fat and vegetable on glycaemia and insulinaemia to a white rice-based meal in healthy adults. Eur J Nutr 2014;53:1719–26.
[34] Hinderliter AL, Babyak MA, Sherwood A, Blumenthal JA. The DASH diet and insulin sensitivity. Curr Hypertens Rep 2011;13:67–73.
[35] Thong FSL, Graham TE. Caffeine-induced impairment of glucose tolerance is abolished by beta-adrenergic receptor blockade in humans. J. Appl. Physiol. Bethesda Md 1985;92:2347–52. 2002.
[36] Graham TE, et al. Caffeine ingestion elevates plasma insulin response in humans during an oral glucose tolerance test. Can J Physiol Pharmacol 2001;79:559–65.
[37] Lovallo WR, Al'absi M, Blick K, Whitsett TL, Wilson MF. Stress-like adrenocorticotropin responses to caffeine in young healthy men. Pharmacol Biochem Behav 1996;55:365–9.
[38] Bellet S, Kershbaum A, Finck EM. Response of free fatty acids to coffee and caffeine. Metabolism 1968;17:702–7.
[39] Cocchi M, Siniscalchi C, Rogato F, Valeriani A. Free fatty acid levels in habitual coffee drinkers in relation to quantities consumed, sex and age. Ann Nutr Metab 1983;27:477–80.
[40] Steinberg D. Fatty acid mobilization–mechanisms of regulation and metabolic consequences. Biochem Soc Symp 1963;24:111–43.
[41] Chen L, Yu M, Shen T, Xia J, Xu BL. Impact of caffeine on β cell proliferation and apoptosis under the influence of palmitic acid. Genet Mol Res 2015;14:5724–30.
[42] Iso H, et al. The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. Ann Intern Med 2006;144:554–62.
[43] Huxley R, et al. Coffee, decaffeinated coffee, and tea consumption in relation to incident type 2 diabetes mellitus: a systematic review with meta-analysis. Arch Intern Med 2009;169:2053–63.
[44] Altmaier E, et al. Metabolomics approach reveals effects of antihypertensives and lipid-lowering drugs on the human metabolism. Eur J Epidemiol 2014;29:325–36.
[45] Gress TW, Nieto FJ, Shahar E, Wofford MR, Brancati FL. Hypertension and antihypertensive therapy as risk factors for type 2 diabetes mellitus. Atherosclerosis Risk in Communities Study. N Engl J Med 2000;342:905–12.
[46] Ferguson DW, Berg WJ, Sanders JS. Clinical and hemodynamic correlates of sympathetic nerve activity in normal humans and patients with heart failure: evidence from direct microneurographic recordings. J Am Coll Cardiol 1990;16:1125–34.
[47] Patel KP. Neural regulation in experimental heart failure. Bailliere Clin Neurol 1997;6:283–96.
[48] Grassi G, Seravalle G, Calhoun DA, Bolla G, Mancia G. Cigarette smoking and the adrenergic nervous system. Clin Exp Hypertens A 1992;14:251–60.
[49] Steptoe A, Ussher M. Smoking, cortisol and nicotine. Int. J. Psychophysiol. Off. J. Int. Organ. Psychophysiol. 2006;59:228–35.
[50] Paulson QX, Hong J, Holcomb VB, Nunez NP. Effects of body weight and alcohol consumption on insulin sensitivity. Nutr J 2010;9:14.
[51] Crabb DW, Liangpunsakul S. Alcohol and lipid metabolism. J Gastroenterol Hepatol 2006;21(Suppl 3):S56–60.