UCSF
UC San Francisco Previously Published Works

Title

Fasting and Postload Nonesterified Fatty Acids and Glucose Dysregulation in Older Adults.

Permalink

https://escholarship.org/uc/item/2jj1z491

Journal

American Journal of Epidemiology, 191(7)

ISSN

0002-9262

Authors

Shitole, Sanyog G
Biggs, Mary L
Ix, Joachim H
et al.

Publication Date

2022-06-27

DOI

10.1093/aje/kwac044

Supplemental Material

https://escholarship.org/uc/item/2jj1z491#supplemental

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

American Journal of Epidemiology
© The Author(s) 2022. Published by Oxford University Press on behalf of the Johns Hopkins Bloomberg School of Public Health. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com.
Vol. 191, No. 7
https://doi.org/10.1093/aje/kwac044
Advance Access publication:
March 8, 2022

Original Contribution

Fasting and Postload Nonesterified Fatty Acids and Glucose Dysregulation in Older Adults

The Cardiovascular Health Study

Sanyog G. Shitole, Mary L. Biggs, Joachim H. Ix, Amanda M. Fretts, Russell P. Tracy, David S. Siscovick, Luc Djoussé, Kenneth J. Mukamal, and Jorge R. Kizer*

* Correspondence to Dr. Jorge R. Kizer, 4150 Clement Street, San Francisco, CA 94121 (e-mail: jorge.kizer@ucsf.edu).

Initially submitted April 28, 2021; accepted for publication March 1, 2022.

To evaluate the association of nonesterified fatty acids (NEFA) with dysglycemia in older adults, NEFA levels were measured among participants in the Cardiovascular Health Study (United States; enrolled 1989–1993). Associations with insulin sensitivity and pancreatic β-cell function, and with incident type 2 diabetes mellitus (DM), were examined. The sample comprised 2,144 participants (aged 77.9 (standard deviation, 4.5) years). Participant data from the Cardiovascular Health Study visit in 1996–1997 was used with prospective follow-up through 2010. Fasting and postload NEFA showed significant associations with lower insulin sensitivity and pancreatic β-cell function, individually and on concurrent adjustment. Over median follow-up of 9.7 years, 236 cases of DM occurred. Postload NEFA were associated with risk of DM (per standard deviation, hazard ratio = 1.18, 95% confidence interval: 1.08, 1.29), but fasting NEFA were not (hazard ratio = 1.12, 95% confidence interval: 0.97, 1.29). The association for postload NEFA persisted after adjustment for putative intermediates, and after adjustment for fasting NEFA. Sex and body mass index modified these associations, which were stronger for fasting NEFA with DM in men but were accentuated for postload NEFA in women and among leaner individuals. Fasting and postload NEFA were related to lower insulin sensitivity and pancreatic β-cell function, but only postload NEFA were associated with increased DM. Additional study into NEFA metabolism could uncover novel potential targets for diabetes prevention in elders.

beta cell function; diabetes; insulin sensitivity; nonesterified fatty acids; older adults

Abbreviations: BMI, body mass index; CHS, Cardiovascular Health Study; CI, confidence interval; CMS, Centers for Medicare and Medicaid Services; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; NEFA, nonesterified fatty acids; OGTT, oral glucose tolerance test; SD, standard deviation.

Disorders of glucose regulation are a major public health concern whose frequency is greatest among older adults (1). Compared with younger adults, the pathophysiology of type 2 diabetes mellitus (DM) in elders is marked by greater contributions from skeletal muscle decline and central fat redistribution, as well as impaired pancreatic β-cell secretory function (2). Given the distinctive features of DM in older adults, and its growing prevalence in the US population (2), improved understanding of its determinants is of foremost importance. Circulating nonesterified fatty acids (NEFA) are a candidate risk factor whose association with incident DM remains incompletely defined, particularly among elders. Circulating NEFA derive mainly from chylomicron spillover postprandially or hydrolysis of triglycerides in the fasting state, reflecting the balance between lipolytic factors, lipolysis inhibitors, and lifestyle factors such as diet, exercise, and social habits (3). Despite the high variability of circulating NEFA levels (3), experimental evidence in humans supports the view that high circulating NEFA promote glucose dysregulation and ultimately incident DM (4). This occurs through impaired insulin signaling in skeletal muscle (5), inhibition of skeletal muscle glucose transport (6), and increased formation of diacylglycerol leading to protein kinase C activation and insulin resistance in liver

Downloaded from https://academic.oup.com/aje/article/191/7/1235/6542790 by UC - San Francisco user on 05 October 2022

and other tissues (7). Together with elevated glucose levels, NEFA also lead to compromised insulin secretory capacity by the β-cells of the pancreas (8).

Several studies demonstrated a positive association between NEFA levels and incident DM (9–12), while one study found this association only in those with impaired glucose tolerance (13). An additional study instead reported an inverse association after adjustment for postload glucose (14), whereas another found a cross-sectional association with metabolic syndrome, but not a longitudinal association with incident DM (15). Inconsistent evidence also exists regarding the relationship of fasting NEFA and pancreatic β-cell dysfunction (16, 17). In our prior work (18), we assessed fasting NEFA in relation to incident DM in older adults, with the association observed only in the first 5 years of follow-up.

Among older adults, postprandial hyperglycemia contributes to DM to a larger extent than in younger adults (2). Postload NEFA levels decline following an oral glucose tolerance test (OGTT), but that suppression in individuals with obesity, and even more so DM, is of lower magnitude than in lean individuals (19). However, the extent to which decreased suppression of NEFA following OGTT, either independently or in conjunction with fasting NEFA, relates to risk of incident DM in elders has not been examined. To address this question, we studied participants from the longitudinal Cardiovascular Health Study (CHS), who completed a 2-hour OGTT to measure fasting and postload NEFA in available specimens. Specifically, we sought to test the hypothesis that fasting and postload NEFA are individually associated with new-onset DM and that the associations persist when they are adjusted for each other.

METHODS

CHS is a prospective investigation that enrolled 5,888 individuals aged 65 years or older from 4 US communities. As previously described (20), participants were selected from randomly generated Medicare-eligible lists in 2 waves: 1989–1990, wherein 5,201 participants were enrolled (original cohort); and 1992–1993, wherein 687 largely Black participants were enrolled (supplemental cohort). Study participants underwent baseline evaluation including standardized questionnaires, physical examination, and laboratory testing. Participants had follow-up every 6 months from 1989 to 1999, alternating between telephone calls and clinic visits. Semiannual telephone calls have continued since. The institutional review board at each center approved the study, and each participant gave informed consent.

For the current analysis, we used data from the 1996–1997 examination, during which an OGTT was administered, as our baseline visit because postload NEFA was a primary exposure of interest. As detailed in Figure 1, of the 5,888 participants who entered the study in 1989–1990 and 1992–1993, 2,144 individuals were available in 1996–1997 for the evaluation of NEFA levels, and 1,987 individuals for the assessment of association between NEFA and incident DM.

Blood specimens were stored continuously at −70°C after collection. Total serum NEFA from fasting and postload samples were measured by the Wako enzymatic method, as previously described (18). The interassay coefficient of variation was 3.54% to 8.17%, with a detectable range of 0.015–1.50 mEq/L.

The primary outcome was incident DM, while secondary cross-sectional outcomes were insulin sensitivity and insulin secretory capacity of pancreatic β-cells. The Matsuda index of insulin sensitivity (21) was calculated from fasting and postload glucose and insulin measures. Second-phase Stumvoll insulin secretory capacity (Stumvoll index) was calculated using a validated formula (22, 23). DM was defined as fasting glucose of ≥126 mg/dL, random glucose of ≥200 mg/dL, or use of antihyperglycemic medication (Web Table 1, available at https://doi.org/10.1093/aje/kwac044). DM was also identified using Centers for Medicare and Medicaid Services (CMS) encounter records and defined as ≥2 inpatient, ≥3 outpatient, or ≥1 inpatient and ≥1 outpatient Medicare claim codes from the *International Classification of Diseases*, *Ninth Revision*, *Clinical Modification*, for DM (prefix 250.xx) over a 2-year period (24). As previously described, glucose measurements were obtained during 1996–1997, 1998–1999, and, in a subset, 2005–2006 (25), mostly on fasting samples. Follow-up for incident DM extended through 2010.

All covariates were measured in 1996–1997, except for diet, assessed in 1995–1996. Race, smoking, and alcohol consumption were self-reported. Trained personnel performed standardized anthropometric determinations. Assessment of physical activity has been reported (25). Heavy alcohol use was defined as >14 drinks/week in men or >7 drinks/week in women. Dietary habits were evaluated by validated food frequency questionnaire (26). Hypertension was defined by systolic and diastolic blood pressure cutoffs of 140 and 90 mm Hg, respectively, or by use of antihypertensive medication. Prevalent heart failure, coronary heart disease, and stroke or transient ischemic attack were ascertained through a combination of CHS questionnaires, medical-record review, and physician confirmation (27, 28). Atrial fibrillation was identified by annual electrocardiograms and diagnostic codes from the *International Classification of Diseases*, *Ninth Revision*, *Clinical Modification*. Estimated glomerular filtration rate (eGFR) was calculated as 76.7 × cystatin $C^{-1.19}$.

We calculated Spearman correlation coefficients for fasting and postload NEFA with continuous variables and median (interquartile range) for different levels of categorical variables and applied the Mann-Whitney-U or Kruskal-Wallis test to assess differences between categories, as appropriate. We used linear or Cox regression to evaluate the association per standard-deviation (SD) increment of NEFA with insulin sensitivity (Matsuda index) and insulin secretion (Stumvoll index) or with incident diabetes after adjustment for covariates. Covariates were chosen based on known biology and previous associations (18, 29, 30). We fitted an initial model adjusting for age, sex and race. We then additionally adjusted for body mass index (BMI), physical activity, smoking status, alcohol consumption, estrogen replacement therapy, serum albumin, and eGFR (main model). A subsequent model additionally adjusted for covariates that could be partial causal intermediates, namely, systolic blood pressure, antihypertensive medication, heart

Downloaded from https://academic.oup.com/aje/article/191/7/1235/6542790 by UC - San Francisco user on 05 October 2022

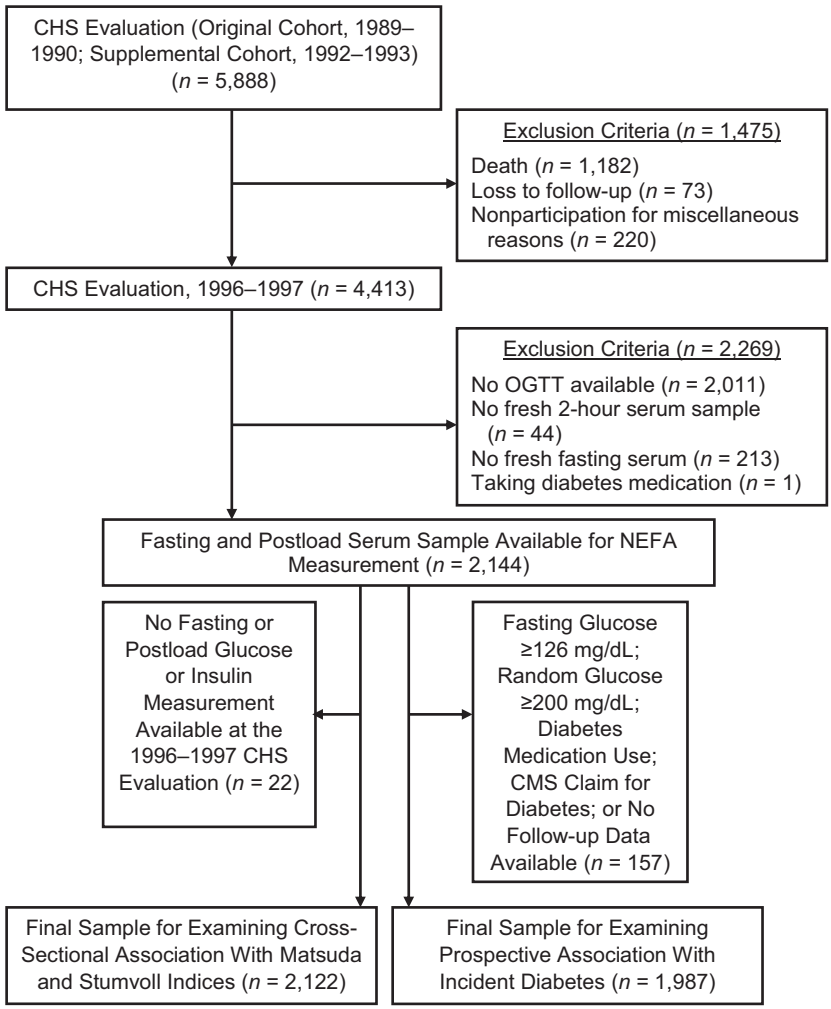

Figure 1. Sample selection flowchart for fasting and postload nonesterified fatty acids (NEFA) and glucose dysregulation in older adults, Cardiovascular Health Study (CHS), United States, 1996–2010. CMS, Centers for Medicare and Medicaid Services; NEFA, nonesterified fatty acids; OGTT, oral glucose tolerance test.

failure, coronary heart disease, stroke or transient ischemic attack, and atrial fibrillation. We also examined associations for all models with concurrent adjustment for fasting and postload NEFA. Exploratory models assessed the impact of further adjustment for dietary habits (carbohydrate, protein, animal fat, vegetable fat, and fiber intake) or, for the primary DM outcome, for fasting and/or postload glucose. In a sensitivity analysis, we restricted follow-up for the DM outcome to the first 5 years. Testing for interaction of fasting and postload NEFA with sex and BMI was performed by inclusion of appropriate cross-product terms in the main models. We used STATA, version 14.2 (StataCorp LP, College Station, Texas) for all analyses. A 2-tailed $P < 0.05$ was considered statistically significant.

RESULTS

The mean age of the study cohort was 77.9 (SD, 4.5) years, with 60% women and 14% Black participants. The baseline characteristics of the cohort, stratified by sex, are presented in Web Table 2. Compared with women, men had higher physical activity, and more common heavy alcohol use, impaired fasting glucose, and prevalent heart failure, coronary heart disease, cerebrovascular event, and atrial fibrillation. Among women, there were more never smokers and greater impaired glucose tolerance than men. Female participants also had higher levels of postload insulin, eGFR, and insulin secretion with lower insulin sensitivity.

The distributions of fasting and postload NEFA are shown in Figure 2. The Spearman correlation coefficient between fasting and postload NEFA was 0.21 ($P < 0.001$). Table 1 presents the associations of fasting and postload NEFA with baseline covariates. Women had a higher fasting NEFA concentration than men, but postload NEFA concentrations were similar between the sexes. Compared with women not on estrogen replacement, those on such therapy had higher fasting, but similar postload, NEFA concentrations. Total cholesterol had a positive correlation with fasting NEFA,

Downloaded from https://academic.oup.com/aje/article/191/7/1235/6542790 by UC - San Francisco user on 05 October 2022

while BMI and waist circumference had positive correlations with postload NEFA concentration.

Table 2 describes the associations of fasting and postload NEFA with the Matsuda and Stumvoll indices in minimal, fully adjusting, and concurrently adjusting linear regression models. Fasting NEFA were inversely associated with insulin sensitivity in the minimally adjusting model (model 1), an association that was modestly attenuated (primarily by BMI) but remained significant in the main model (model 2) and persisted after adjustment for potential causal intermediates (model 3). Similarly, postload NEFA were inversely associated with insulin sensitivity in the minimally adjusting and, after modest attenuation (mostly by BMI), in the main model, with persistence in the final model including possible causal intermediates. In the models adjusting concurrently for fasting and postload NEFA, modest weakening of the associations was observed for each, but both fasting and postload NEFA remained significantly inversely associated with insulin sensitivity at all levels of adjustment. The association for fasting NEFA was numerically stronger than for postload NEFA, but the 95% confidence intervals (CIs) overlapped substantially. Fasting and postload NEFA were inversely associated with insulin secretion in minimally adjusting models, associations that strengthened after additional adjustment for possible confounders (again, mainly BMI) in the main model, and remained after further adjustment for potential causal intermediates. In the models adjusting jointly for fasting and postload NEFA, there was modest weakening of the associations. The relationship was numerically stronger for fasting than for postload NEFA, but 95% CIs again overlapped. Additional adjustment for dietary intake measures had no meaningful influence on the associations for fasting or postload NEFA (data not shown).

Over a median follow-up of 9.7 years (range, 0.01–13.6), we documented 236 cases of incident DM (147 in women, 89 in men). Figure 3 describes the associations of fasting and postload NEFA with incident DM individually and jointly at different levels of adjustment in Cox models. Fasting NEFA exhibited a borderline significant association in the minimal model (adjusting for demographic factors), but the association was slightly attenuated and became nonsignificant at higher levels of adjustment (models 2 and 3). Specifically, in the main model (model 2), for each SD increment in fasting NEFA, there was a nonsignificant 11% (95% CI: −3, 29) increase in risk of incident DM. By contrast, postload NEFA showed a significant association in the minimally adjusting model, with risk estimates that did not materially change on progressive adjustment in the higher-order models. In the main model, each SD increment in postload NEFA was associated with an 18% (8%–29%) increase in risk of incident DM. Upon adjustment for postload NEFA, the association between fasting NEFA and incident DM weakened, and it was nonsignificant across all models. In turn, adjustment for fasting NEFA had no material impact on the association between postload NEFA and incident DM, which remained significant at all levels of adjustment. These findings were similar when follow-up was limited to 5 years. Further adjustment for dietary intake measures did not meaningfully alter the associations (data not shown). In an additional exploratory analysis that further adjusted fasting NEFA for fasting glucose, the association remained null (hazard ratio = 1.02 per SD, 95% CI: 0.88, 1.18; P = 0.80). In the case of postload NEFA, additional adjustment for fasting and postload glucose attenuated the association and rendered it nonsignificant (hazard ratio = 1.07 per SD, 95% CI: 0.95, 1.21; P = 0.24).

As detailed in Table 3 and Web Figures 1 and 2, we found evidence of interaction of fasting and postload NEFA by sex and/or BMI. There was effect modification by sex for the associations of both fasting and postload NEFA with the Matsuda index, but the direction of interaction differed. For fasting NEFA, the inverse association with insulin sensitivity was stronger in men than women. By contrast, for postload NEFA, the inverse association with insulin sensitivity was stronger in women than men. Effect modification of fasting and postload NEFA by sex was also present in relation to the Stumvoll index. Yet the inverse association of NEFA with insulin secretion was stronger for men than women in both the fasting and postload state. In the case of incident DM, there was again evidence of effect modification by sex for both fasting and postload NEFA, but the pattern mirrored that for insulin sensitivity. For fasting NEFA, the relationship with incident DM was observed in men but not in women, whereas for postload NEFA, the association was stronger in women than men. Interactions with BMI were detected for both fasting and postload NEFA in relation to the Matsuda index. The inverse associations were stronger with lower BMI, both for fasting and postload NEFA, although they did not exhibit the same progressive decline across standard BMI categories in the case of postload NEFA. There was no evidence of interaction, however, between NEFA and BMI in relation to the Stumvoll index. For DM, effect modification by BMI was observed for postload, but not for fasting NEFA, with a stronger association of postload NEFA with DM at lower BMI.

DISCUSSION

In this study of elders, fasting and postload NEFA were inversely associated with insulin sensitivity and secretion independent of potential confounders and partial mediators when assessed individually, as well as jointly. Postload NEFA alone, and not fasting NEFA, were significantly associated with incident DM, a relationship that persisted after adjustment for potential confounding factors, possible mediators, and also fasting NEFA. There was evidence of effect modification of NEFA associations with glucose dysregulation outcomes by sex and BMI. For fasting NEFA, an association with incident DM emerged for men, whereas for postload NEFA, the observed association was stronger in women, as well as at lower adiposity.

Increased NEFA levels are recognized to have dysmetabolic effects by inhibition of glucose uptake and oxidation (4), and to reduce insulin-mediated inhibition of hepatic glucose output (31). Elevated NEFA also result in increased generation of lipid mediators such as ceramide and diacylglycerol (32–34), which have been implicated not only in hepatic insulin resistance (35), but also in impairment of pancreatic β-cells (36). Furthermore, high NEFA levels

Downloaded from https://academic.oup.com/aje/article/191/7/1235/6542790 by UC - San Francisco user on 05 October 2022

Table 1. Associations of Baseline Characteristics With Fasting and Postload Nonesterified Fatty Acids in Older Adults, Cardiovascular Health Study, United States, 1996–1997

Characteristic	No.[a]	%[a]	Fasting NEFA				Postload NEFA			
			Spearman's ρ	*P* Value	Median (IQR) mEq/L	*P* Value	Spearman's ρ	*P* Value	Median (IQR) mEq/L	*P* Value
Age			0.07	<0.001			−0.05	0.037		
Sex						<0.001				0.093
Female	1,294	60.4			0.40 (0.29, 0.51)				0.05 (0.04, 0.07)	
Male	850	39.6			0.28 (0.20, 0.37)				0.05 (0.04, 0.08)	
Race						0.956				0.222
Non-Black	1,843	86.0			0.34 (0.25, 0.46)				0.05 (0.04, 0.07)	
Black	301	14.0			0.34 (0.25, 0.45)				0.05 (0.04, 0.07)	
BMI[b]			0.06	0.006			0.17	<0.001		
Waist circumference			0.02	0.418			0.18	<0.001		
Physical activity			−0.11	<0.001			−0.02	0.466		
Smoking status						<0.001				<0.001
Never	934	43.6			0.36 (0.27, 0.50)				0.05 (0.04, 0.07)	
Former	1,060	49.4			0.33 (0.24, 0.44)				0.05 (0.04, 0.07)	
Current	150	7.0			0.31 (0.22, 0.41)				0.04 (0.03, 0.06)	
Heavy alcohol use						0.042				0.002
No	1,958	91.6			0.34 (0.25, 0.46)				0.05 (0.04, 0.07)	
Yes	180	8.4			0.37 (0.26, 0.48)				0.04 (0.03, 0.07)	
Estrogen replacement therapy (women)						<0.001				<0.001
No	1,048	81.0			0.38 (0.28, 0.50)				0.05 (0.03, 0.07)	
Yes	246	19.0			0.44 (0.31, 0.56)				0.06 (0.04, 0.08)	
Dietary intake										
Carbohydrates			−0.02	0.40			−0.01	0.75		
Protein			−0.04	0.05			−0.01	0.69		
Vegetable fat			−0.05	0.02			−0.02	0.42		
Animal fat			−0.04	0.08			0.06	0.01		
Fiber			−0.03	0.20			−0.04	0.08		
Hypertension						<0.001				0.126
No	857	40.1			0.32 (0.23, 0.44)				0.05 (0.04, 0.07)	
Yes	1,280	59.9			0.36 (0.27, 0.48)				0.05 (0.04, 0.07)	

Table continues

Downloaded from https://academic.oup.com/aje/article/191/7/1235/6542790 by UC - San Francisco user on 05 October 2022

Table 1. Continued

Characteristic	No.[a]	%[a]	Fasting NEFA				Postload NEFA			
			Spearman's ρ	*P* Value	Median (IQR) mEq/L	*P* Value	Spearman's ρ	*P* Value	Median (IQR) mEq/L	*P* Value
Total cholesterol			0.17	<0.001			0.05	0.027		
Heart failure						0.325				0.831
No (*n* = 2007)	2,007	93.6			0.34 (0.25, 0.46)				0.05 (0.04, 0.07)	
Yes (*n* = 137)	137	6.4			0.36 (0.27, 0.46)				0.05 (0.04, 0.07)	
CHD						<0.001				0.308
No	1,687	78.7			0.35 (0.26, 0.47)				0.05 (0.04, 0.07)	
Yes	457	21.3			0.32 (0.22, 0.43)				0.05 (0.04, 0.07)	
Stroke/TIA						0.093				0.780
No (*n* = 1,976)	1,976	92.2			0.34 (0.25, 0.46)				0.05 (0.04, 0.07)	
Yes (*n* = 168)	168	7.8			0.37 (0.27, 0.49)				0.05 (0.03, 0.08)	
Atrial fibrillation						0.372				0.187
No (*n* = 2,056)	2,056	96.8			0.34 (0.25, 0.46)				0.05 (0.04, 0.07)	
Yes (*n* = 69)	69	3.2			0.37 (0.25, 0.54)				0.04 (0.03, 0.07)	
Fasting glucose			0.08	<0.001			0.23	<0.001		
Postload glucose			0.37	<0.001			0.30	<0.001		
Fasting insulin			0.08	<0.001			0.32	<0.001		
Postload insulin			0.22	<0.001			0.25	<0.001		
Albumin			0.07	0.001			0.09	<0.001		
eGFR			−0.003	0.875			−0.09	<0.001		

Abbreviations: BMI, body mass index; CHD, coronary heart disease; eGFR, estimated glomerular filtration rate; IQR, interquartile range; NEFA, nonesterified fatty acids; TIA, transient ischemic attack.

[a] For categorical variables.

[b] BMI is calculated as weight (kg)/height $(m)^2$.

Downloaded from https://academic.oup.com/aje/article/191/7/1235/6542790 by UC - San Francisco user on 05 October 2022

Table 2. Association of Fasting and Postload Nonesterified Fatty Acids With Matsuda Index and Stumvoll Index of Insulin Secretion in Older Adults, Cardiovascular Health Study, United States, 1996–1997

Exposure[a]	Model 1[b]			Model 2[c]			Model 3[d]		
	MD	95% CI	*P* Value	MD	95% CI	*P* Value	MD	95% CI	*P* Value
Matsuda index									
Fasting NEFA[e]	−0.49	−0.58, −0.39	<0.001	−0.41	−0.50, −0.32	<0.001	−0.42	−0.51, −0.33	<0.001
Postload NEFA[f]	−0.39	−0.48, −0.30	<0.001	−0.29	−0.37, −0.20	<0.001	−0.29	−0.37, −0.20	<0.001
Fasting NEFA[e] (adjusting for postload NEFA)	−0.41	−0.50, −0.31	<0.001	−0.36	−0.46, −0.27	<0.001	−0.37	−0.46, −0.27	<0.001
Postload NEFA[f] (adjusting for fasting NEFA)	−0.29	−0.38, −0.20	<0.001	−0.21	−0.30, −0.13	<0.001	−0.21	−0.30, −0.13	<0.001
Stumvoll index of insulin secretion									
Fasting NEFA[e]	−16.5	−24.3, −8.7	<0.001	−22.6	−30.2, −15.0	<0.001	−21.8	−29.5, −14.2	<0.001
Postload NEFA[f]	−7.1	−14.4, 0.2	0.058	−14.4	−21.5, −7.3	<0.001	−14.0	−21.1, −6.9	<0.001
Fasting NEFA[e] (adjusting for postload NEFA)	−15.6	−23.6, −7.6	<0.001	−20.1	−27.9, −12.4	<0.001	−19.4	−27.3, −11.6	<0.001
Postload NEFA[f] (adjusting for fasting NEFA)	−3.5	−11.0, 4.1	0.366	−10.2	−17.4, −3.0	0.006	−10.1	−17.3, −2.8	0.006

Abbreviations: CI, Confidence interval; MD, mean difference; NEFA, nonesterified fatty acids.

[a] Mean differences are calculated per standard-deviation increment in NEFA.

[b] Model 1 adjusted for age, sex, and race.

[c] Model 2 adjusted for covariates in model 1, body mass index, physical activity, smoking status, alcohol consumption, estrogen use, serum albumin, and estimated glomerular filtration rate.

[d] Model 3 adjusted for covariates in model 2, systolic blood pressure, antihypertension medications, prevalent heart failure, prevalent coronary heart disease, prevalent stroke or transient ischemic attack, and prevalent atrial fibrillation.

[e] Standard deviation of fasting NEFA = 0.16 mEq/L.

[f] Standard deviation of postload NEFA = 0.045 mEq/L.

Downloaded from https://academic.oup.com/aje/article/191/7/1235/6542790 by UC - San Francisco user on 05 October 2022

Table 3. Interactions of Fasting and Postload Nonesterified Fatty Acids With Sex and Body Mass Index in Relation to Abnormal Glucose Metabolism in Older Adults, Cardiovascular Health Study, United States, 1996–2010

Exposure[a,b]	Matsuda Index			Stumvoll Index of Insulin Secretion			Incident Diabetes		
	P for Interaction	MD	95% CI	*P* for Interaction	MD	95% CI	*P* for Interaction	HR	95% CI
Fasting NEFA[c]									
Sex	0.049			0.003			0.01		
Female		−0.36	−0.46, −0.25		−15.2	−24.2, −6.2		1.00	0.85, 1.19
Male		−0.55	−0.71, −0.31		−39.2	−52.7, −25.8		1.48	1.15, 1.90
Body mass index[d]	<0.001			0.28			0.34		
<25.0		−0.66	−0.80, −0.52		−28.0	−39.6, −16.4		1.15	0.89, 1.49
25.0–29.9		−0.37	−0.50, −0.24		−19.1	−30.1, −8.1		1.13	0.92, 1.38
≥30.0		−0.04	−0.24, 0.17		−19.1	−36.2, −1.9		1.08	0.84, 1.38
Postload NEFA[e]									
Sex	<0.001			0.003			0.03		
Female		−0.44	−0.55, −0.32		−4.6	−14.2, 5.0		1.39	1.18, 1.63
Male		−0.12	−0.24, 0.01		−25.7	−35.9, −15.4		1.12	0.99, 1.26
Body mass index[d]	<0.001			0.36			0.02		
<25.0		−0.32	−0.46, −0.19		−12.1	−23.4, −0.8		1.47	0.14, 1.63
25.0–29.9		−0.56	−0.70, −0.42		−20.2	−32.2, −8.3		1.29	1.06, 1.56
≥30.0		0.13	−0.03, 0.29		−10.5	−24.2, 3.1		1.13	1.00, 1.27

Abbreviations: CI, confidence interval; HR, hazard ratio; MD, mean difference; NEFA, nonesterified fatty acids.
[a] All models adjusted for age, sex, race, body mass index, physical activity, smoking status, alcohol consumption, estrogen use, serum albumin, and estimated glomerular filtration rate.
[b] Risk estimates are per standard deviation of NEFA.
[c] Standard deviation of fasting NEFA = 0.16 mEq/L.
[d] *P* for interaction is for continuous body mass index, calculated as weight (kg)/height $(m)^2$.
[e] Standard deviation of postload NEFA = 0.045 mEq/L.

Downloaded from https://academic.oup.com/aje/article/191/7/1235/6542790 by UC - San Francisco user on 05 October 2022

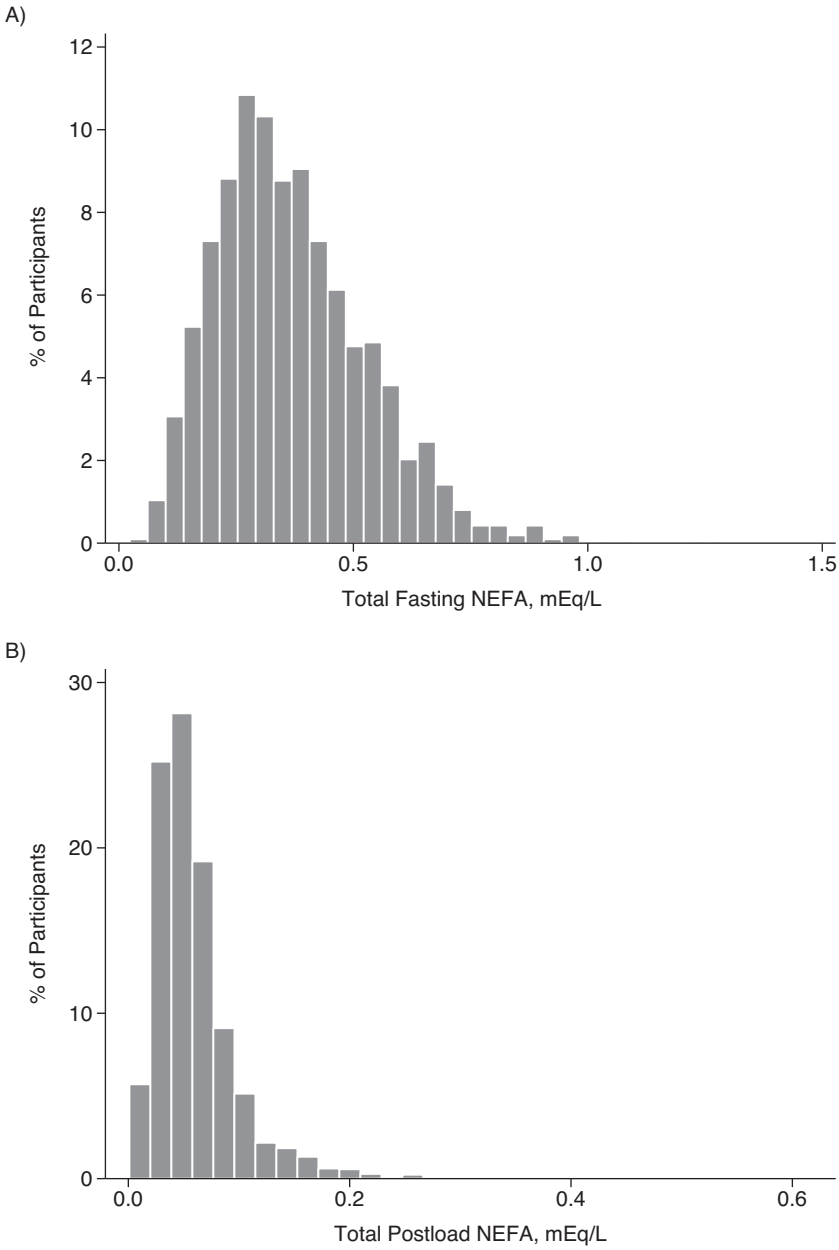

Figure 2. Distribution of fasting and postload nonesterified fatty acids (NEFA) in older adults, United States, Cardiovascular Health Study, 1996–1997. Median (minimum, maximum) for fasting and postload NEFA was 0.35 (0.02, 1.29) and 0.05 (0.001, 0.63) mEq/L respectively.

induce inflammation (37, 38), a well-established mediator of insulin resistance and pancreatic β-cell damage (39), providing an additional causal link between NEFA and DM.

Longitudinal cohort studies have also examined the association between NEFA and glucose dysregulation. A study in Pima Indians concluded that large fat cells resulted in increased fasting NEFA, both of which were associated with increased DM risk (11), while a French study found high fasting NEFA to predict deterioration of glucose tolerance (9). Using fasting NEFA measured at the 1992–1993 CHS visit, our group also found an association between NEFA and incident DM at 5-year follow-up but not beyond (18). The Atherosclerosis Risk in Communities study also reported a modest but significant association between fasting NEFA and incident DM in its middle-aged participants (10). In contrast, another study of young/middle-aged adults concluded that higher fasting and postload NEFA levels are a response to, rather than a predisposing factor for, metabolic syndrome or DM (15). In turn, the Insulin Resistance Atherosclerosis Study (14) reported that adjustment for postload glucose altered the association between fasting NEFA and DM from positive to inverse.

Downloaded from https://academic.oup.com/aje/article/191/7/1235/6542790 by UC - San Francisco user on 05 October 2022

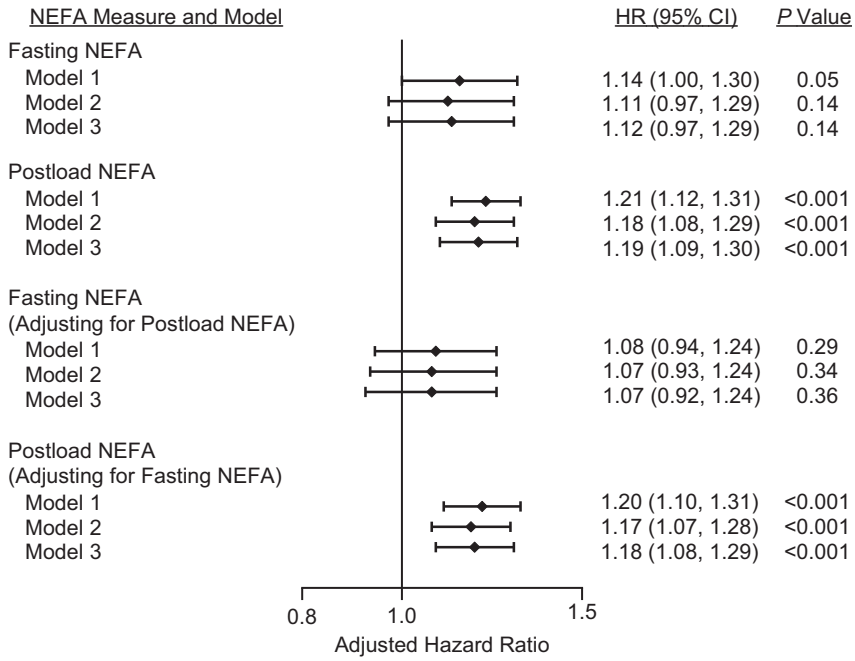

Figure 3. Hazard ratios for incident diabetes per standard deviation (SD) of fasting and postload nonesterified fatty acids (NEFA) in older adults, Cardiovascular Health Study, United States, 1996–2010. SD of fasting NEFA = 0.16 mEq/L. SD of postload NEFA = 0.045 mEq/L. Model 1 adjusted for age, sex, and race; model 2 adjusted for covariates in model 1, body mass index, physical activity, smoking status, alcohol consumption, estrogen replacement therapy, serum albumin, and estimated glomerular filtration rate; model 3 adjusted for covariates in model 2, systolic blood pressure, antihypertension medications, prevalent heart failure, prevalent coronary heart disease, prevalent stroke or transient ischemic attack, and prevalent atrial fibrillation. CI, confidence interval; HR, hazard ratio.

None of the prior prospective examinations included measurements of postload NEFA in older adults. One small study of women showed stronger correlations of postload than fasting NEFA with markers of obesity, inflammation, and insulin resistance (40). In our larger and older cohort, we did not observe the same variation in our cross-sectional analysis, where the mean difference in insulin sensitivity per SD of fasting NEFA was instead higher in absolute value than for postload NEFA.

The present study is the first to evaluate postload NEFA, alongside fasting NEFA, and incident DM in an older cohort. Our finding that postload NEFA were associated with incident DM independently of fasting NEFA in elders is therefore novel. The nonsignificant association for fasting NEFA at the 1996–1997 exam contrasts with the previous association with diabetes, albeit only in the near term, documented for fasting NEFA in the 1992–1993 exam with a mean follow-up of 9.5 years (18). The earlier analysis had a larger sample size and >20% more events, however, such that this could reflect a difference in power. Unlike the previous study, which did not detect interaction with sex, the present analyses did show such evidence, with a significant association between fasting NEFA and DM in men but not women. Evaluation of incident DM events using CMS data was not available for the earlier analyses (18), and when the 1992–1993 exam data was evaluated for interaction by sex accounting for the additional incident DM events from the CMS data, we found higher hazard ratios for men compared with women per SD of fasting NEFA, which was similar to the present findings. The present results also suggest that mechanisms governing postload NEFA handling may have greater implications for diabetes pathogenesis in older adults than fasting NEFA levels.

In healthy individuals, fasting NEFA are higher than postload NEFA levels, decreasing markedly at 2 hours during an OGTT (40). Elevated postload or postprandial NEFA are a manifestation of ineffective suppression of adipose tissue lipolysis linked to obesity and insulin resistance (19). Regulation of circulating NEFA levels resides primarily in adipose tissue, but differs in the fasting and postprandial states (41). Following a meal, circulating NEFA levels are determined by the balance between activity of lipoprotein lipase (LPL) in adipose tissue capillaries, which effects hydrolysis of triglycerides in chylomicron or very low-density lipoprotein (VLDL) particles, and re-esterification of the released NEFA within adipose cells (4). In our study, such regulation would apply specifically to VLDL, and not chylomicrons, since lipids were not ingested. By contrast, during the fasting state, bloodstream NEFA levels are driven by lipolysis of adipose tissue triglycerides by adipose triglyceride lipase and hormone-sensitive lipase (41). In addition, LPL in adipose endothelium is inhibited by angiopoietin-like 3, whose levels decrease postprandially, and by angiopoietin-like 4, whose levels are maximally expressed in the postabsorptive state (42). The distinct regulatory components that determine circulating NEFA in the

Downloaded from https://academic.oup.com/aje/article/191/7/1235/6542790 by UC - San Francisco user on 05 October 2022

fed and fasting states could potentially contribute to the differences in diabetes risk observed for postload and fasting NEFA.

There are also several potential mechanisms through which higher postload or postprandial NEFA levels may exert more deleterious effects on glucose metabolism than fasting NEFA levels. Greater delivery of NEFA to the liver after a glucose load or meal provides higher substrate for generation of acetyl coenzyme A, which apart from helping to drive greater hepatic fatty acid synthesis and very low-density lipoprotein (VLDL) secretion postprandially, is an important positive regulator of gluconeogenesis (4). Hence, postprandial NEFA would drive higher postprandial glucose levels at a time when hepatic gluconeogenesis should be suppressed. Whether as a result of NEFA-driven gluconeogenesis or peripheral (e.g., skeletal muscle) insulin resistance, such higher postprandial glucose levels in tandem with high NEFA levels could accentuate injurious effects on pancreatic β-cells, as evidence favors their combination (glucolipotoxicity) and not high NEFA alone (lipotoxicity) as the operative mechanism in vivo (8). In addition, higher circulating VLDL increases delivery of NEFA to adipose tissue, resulting in or compounding adipocyte hypertrophy and insulin resistance (4). Adipose-tissue resistance to insulin-mediated glucose uptake, a key determinant of de novo lipogenesis in adipocytes that leads to synthesis of branched fatty acid esters of hydroxy fatty acids, also reduces production of these compounds and their attendant insulin-sensitizing properties in adipose tissue and liver (43).

In exploratory analyses, the significant association observed for postload NEFA and incident DM was abolished by adjustment for fasting and postload glucose. Adjustment for glucose may represent overadjustment, however, since it is the basis for the definition of diabetes. To the extent that glucose levels may be downstream of NEFA (although the relationship is admittedly complex and interdependent), adjustment for glucose could mask a relevant influence of postload NEFA on development of diabetes. Hence, while an earlier study (14) considered postload glucose a confounder of the NEFA-DM relationship, we believe that attenuation of the relationship in the present study does not negate a biologically meaningful impact of postload NEFA on DM risk. To be sure, these findings indicate that postload NEFA may not be useful for DM risk prediction over and above glucose. However, our findings are important because they suggest that better understanding of the molecular mechanisms regulating postload NEFA levels could potentially uncover novel therapeutic targets to reduce DM risk in older adults.

Regarding the observed NEFA-by-sex interactions, sex differences in the biology of glucose homeostasis have been described (44). The strong association of fasting NEFA with DM seen in men, but not women, is consistent with the pattern observed in our prior study, although testing for interaction was not significant at that earlier time point (18). The male predilection observed here also accorded with more robust inverse associations of fasting NEFA with insulin sensitivity and secretion in this group. By contrast, the more robust association of postload NEFA with DM in female versus male participants was aligned with their stronger inverse relationship with insulin sensitivity but not with their weaker inverse relationship with insulin secretion. It has been documented that although NEFA reduce insulin suppression of hepatic glucose production in older adults of both sexes, they impair peripheral glucose disposal in older men but not older women (44). Hence, a potential explanation for the robust relationship of fasting NEFA with DM in men could involve NEFA's male-specific impact on skeletal muscle insulin resistance. The more robust association of postload NEFA with DM in women is harder to explain, but it could signal a particular decline in female adipose tissue's greater capacity for triglyceride storage, and heightened adverse consequences to liver and pancreas of increased post-OGTT fatty acid spillover. Indeed, the parallel finding that postload NEFA showed a stronger relationship with DM at lower BMI suggests that, in the setting of diminished adipose-tissue content, the implications of high postload NEFA levels for DM risk are especially prominent.

Our study has several limitations. One-time measurements of NEFA are known to be unreliable owing to the high variability NEFA flux and NEFA's short half-life (3). Repeated measurements would help address this variability, but this was not available for postload NEFA. Thus, our single measurements of NEFA may be underestimating the associations with longer-term events. Still, when we conducted the incident DM analysis restricting follow-up to 5 years, the results for fasting and postload NEFA were unchanged. We did not measure individual NEFA species or classes in both the fasting and postload state and are unable to evaluate their specific contributions to our findings here. Last, returning CHS participants who underwent OGTT during the 1996–1997 exam were a healthy subset of the cohort (45). Accordingly, our findings may not be applicable to all older adults, nor are they necessarily generalizable to younger adults or to other racial-ethnic groups.

In conclusion, in this older cohort, both fasting and postload NEFA were associated with lower insulin sensitivity and β-cell function, but only postload NEFA were associated with incident DM. Our findings provide impetus for further investigation of fasting and postload NEFA in relation to new-onset DM in older adults, including replication of the observed sex/BMI differences and their basis. Study of the mechanisms that might account for this closer relationship of postload NEFA with new-onset DM late in life could illuminate novel approaches to prevention in this highly susceptible segment of the population.

ACKNOWLEDGMENTS

Author affiliations: Cardiology Section, San Francisco Veterans Affairs Health Care System, San Francisco, California, United States (Sanyog G. Shitole, Jorge R. Kizer); Department of Medicine, School of Medicine, University of California San Francisco, San Francisco, California, United States (Sanyog G. Shitole, Jorge R. Kizer); Department of Biostatistics, University of Washington, Seattle, Washington, United States (Mary L.

Downloaded from https://academic.oup.com/aje/article/191/7/1235/6542790 by UC - San Francisco user on 05 October 2022

Biggs); Department of Medicine, School of Medicine, University of California San Diego, La Jolla, California, United States (Joachim H. Ix); Department of Epidemiology, School of Public Health, University of Washington, Seattle, Washington, United States (Amanda M. Fretts); Department of Pathology and Laboratory Medicine, Larner College of Medicine, University of Vermont, Burlington, Vermont, United States (Russell P. Tracy); New York Academy of Medicine, New York, New York, United States (David S. Siscovick); Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, United States (Luc Djoussé); and Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts, United States (Kenneth J. Mukamal).

This research was supported by the National Heart, Lung, and Blood Institute (contracts HHSN268201200036C, HHSN268200800007C, HHSN268201800001C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and 75N92021D00006 and grants U01HL080295 and U01HL130114), with additional contribution from the National Institute of Neurological Disorders and Stroke. Support was also provided by the National Institute on Aging (primary: R01AG053325, additional: R01AG023629). Additional support also provided by the National Heart, Lung, and Blood Institute (grant K24HL135413).

Study protocol is available upon request. Statistical code is not available. Data set is not available from the authors. Interested readers can review the Cardiovascular Health Study procedures for outside investigators to obtain and analyze data (https://chs-nhlbi.org/CHSData.htm).

A full list of principal Cardiovascular Health Study investigators and institutions can be found at https://chs-nhlbi.org/pi.

The views expressed in this article are those of the authors and do not reflect those of the National Institutes of Health.

J.R.K. reports stock ownership in Abbott, Bristol-Myers Squibb, Johnson & Johnson, Medtronic, Merck, and Pfizer. The other authors report no conflicts.

REFERENCES

1. Menke A, Casagrande S, Geiss L, et al. Prevalence of and trends in diabetes among adults in the United States, 1988–2012. *JAMA*. 2015;314(10):1021–1029.
2. Lee PG, Halter JB. The pathophysiology of hyperglycemia in older adults: clinical considerations. *Diabetes Care*. 2017; 40(4):444–452.
3. Karpe F, Dickmann JR, Frayn KN. Fatty acids, obesity, and insulin resistance: time for a reevaluation. *Diabetes*. 2011; 60(10):2441–2449.
4. Yang A, Mottillo EP. Adipocyte lipolysis: from molecular mechanisms of regulation to disease and therapeutics. *Biochem J*. 2020;477(5):985–1008.
5. Wilding JP. The importance of free fatty acids in the development of type 2 diabetes. *Diabet Med*. 2007;24(9): 934–945.
6. Griffin ME, Marcucci MJ, Cline GW, et al. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. *Diabetes*. 1999;48(6):1270–1274.
7. Jornayvaz FR, Shulman GI. Diacylglycerol activation of protein kinase Cε and hepatic insulin resistance. *Cell Metab*. 2012;15(5):574–584.
8. Prentki M, Peyot ML, Masiello P, et al. Nutrient-induced metabolic stress, adaptation, detoxification, and toxicity in the pancreatic β-cell. *Diabetes*. 2020;69(3):279–290.
9. Charles MA, Eschwège E, Thibult N, et al. The role of non-esterified fatty acids in the deterioration of glucose tolerance in Caucasian subjects: results of the Paris Prospective Study. *Diabetologia*. 1997;40(9):1101–1106.
10. Pankow JS, Duncan BB, Schmidt MI, et al. Fasting plasma free fatty acids and risk of type 2 diabetes: the Atherosclerosis Risk in Communities study. *Diabetes Care*. 2004;27(1):77–82.
11. Paolisso G, Tataranni PA, Foley JE, et al. A high concentration of fasting plasma non-esterified fatty acids is a risk factor for the development of NIDDM. *Diabetologia*. 1995;38(10):1213–1217.
12. Salgin B, Ong KK, Thankamony A, et al. Higher fasting plasma free fatty acid levels are associated with lower insulin secretion in children and adults and a higher incidence of type 2 diabetes. *J Clin Endocrinol Metab*. 2012;97(9): 3302–3309.
13. Stefan N, Stumvoll M, Bogardus C, et al. Elevated plasma nonesterified fatty acids are associated with deterioration of acute insulin response in IGT but not NGT. *Am J Physiol Endocrinol Metab*. 2003;284(6):E1156–E1161.
14. Il'yasova D, Wang F, D'Agostino RB Jr, et al. Prospective association between fasting NEFA and type 2 diabetes: impact of post-load glucose. *Diabetologia*. 2010;53(5): 866–874.
15. Byrne CD, Maison P, Halsall D, et al. Cross-sectional but not longitudinal associations between non-esterified fatty acid levels and glucose intolerance and other features of the metabolic syndrome. *Diabet Med*. 1999;16(12):1007–1015.
16. Johnston LW, Harris SB, Retnakaran R, et al. Association of NEFA composition with insulin sensitivity and beta cell function in the Prospective Metabolism and Islet Cell Evaluation (PROMISE) cohort. *Diabetologia*. 2018;61(4): 821–830.
17. Rebelos E, Seghieri M, Natali A, et al. Influence of endogenous NEFA on beta cell function in humans. *Diabetologia*. 2015;58(10):2344–2351.
18. Djousse L, Khawaja O, Bartz TM, et al. Plasma fatty acid-binding protein 4, nonesterified fatty acids, and incident diabetes in older adults. *Diabetes Care*. 2012;35(8): 1701–1707.
19. Boston RC, Moate PJ. NEFA minimal model parameters estimated from the oral glucose tolerance test and the meal tolerance test. *Am J Physiol Regul Integr Comp Physiol*. 2008;295(2):R395–R403.
20. Fried LP, Borhani NO, Enright P, et al. The Cardiovascular Health Study: design and rationale. *Ann Epidemiol*. 1991; 1(3):263–276.
21. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care*. 1999;22(9): 1462–1470.

Downloaded from https://academic.oup.com/aje/article/191/7/1235/6542790 by UC - San Francisco user on 05 October 2022

22. Stumvoll M, Van Haeften T, Fritsche A, et al. Oral glucose tolerance test indexes for insulin sensitivity and secretion based on various availabilities of sampling times. *Diabetes Care*. 2001;24(4):796–797.
23. Stumvoll M, Mitrakou A, Pimenta W, et al. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. *Diabetes Care*. 2000;23(3):295–301.
24. Brutsaert EF, Biggs ML, Delaney JA, et al. Longitudinal assessment of *N*-terminal pro-B-type natriuretic peptide and risk of diabetes in older adults: the Cardiovascular Health Study. *Metabolism*. 2016;65(10):1489–1497.
25. Kizer JR, Arnold AM, Benkeser D, et al. Total and high-molecular-weight adiponectin and risk of incident diabetes in older people. *Diabetes Care*. 2012;35(2):415–423.
26. Feskanich D, Rimm EB, Giovannucci EL, et al. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. *J Am Diet Assoc*. 1993;93(7):790–796.
27. Ives DG, Fitzpatrick AL, Bild DE, et al. Surveillance and ascertainment of cardiovascular events. The Cardiovascular Health Study. *Ann Epidemiol*. 1995;5(4):278–285.
28. Psaty BM, Kuller LH, Bild D, et al. Methods of assessing prevalent cardiovascular disease in the Cardiovascular Health Study. *Ann Epidemiol*. 1995;5(4):270–277.
29. Djoussé L, Benkeser D, Arnold A, et al. Plasma free fatty acids and risk of heart failure: the Cardiovascular Health Study. *Circ Heart Fail*. 2013;6(5):964–969.
30. Djoussé L, Biggs ML, Lemaitre RN, et al. Plasma omega-3 fatty acids and incident diabetes in older adults. *Am J Clin Nutr*. 2011;94(2):527–533.
31. Boden G, Chen X, Ruiz J, et al. Mechanisms of fatty acid-induced inhibition of glucose uptake. *J Clin Invest*. 1994;93(6):2438–2446.
32. Adams JM 2nd, Pratipanawatr T, Berria R, et al. Ceramide content is increased in skeletal muscle from obese insulin-resistant humans. *Diabete*. 2004;53(1):25–31.
33. Watt MJ, Barnett AC, Bruce CR, et al. Regulation of plasma ceramide levels with fatty acid oversupply: evidence that the liver detects and secretes de novo synthesised ceramide. *Diabetologia*. 2012;55(10):2741–2746.
34. Zhang C, Klett EL, Coleman RA. Lipid signals and insulin resistance. *Clin Lipidol*. 2013;8(6):659–667.
35. Samuel VT, Shulman GI. Nonalcoholic fatty liver disease, insulin resistance, and ceramides. *N Engl J Med*. 2019; 381(19):1866–1869.
36. Carpentier A, Mittelman SD, Bergman RN, et al. Prolonged elevation of plasma free fatty acids impairs pancreatic beta-cell function in obese nondiabetic humans but not in individuals with type 2 diabetes. *Diabetes*. 2000;49(3): 399–408.
37. Liang H, Tantiwong P, Sriwijitkamol A, et al. Effect of a sustained reduction in plasma free fatty acid concentration on insulin signalling and inflammation in skeletal muscle from human subjects. *J Physiol*. 2013;591(11):2897–2909.
38. Tripathy D, Mohanty P, Dhindsa S, et al. Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. *Diabetes*. 2003;52(12)): 2882–2887.
39. Shitole SG, Biggs ML, Reiner AP, et al. Soluble CD14 and CD14 variants, other inflammatory markers, and glucose dysregulation in older adults: the Cardiovascular Health Study. *Diabetes Care*. 2019;42(11):2075–2082.
40. Karakas SE, Almario RU, Kim K. Serum fatty acid binding protein 4, free fatty acids, and metabolic risk markers. *Metabolism*. 2009;58(7):1002–1007.
41. Lafontan M, Langin D. Lipolysis and lipid mobilization in human adipose tissue. *Prog Lipid Res*. 2009;48(5):275–297.
42. Minicocci I, Tikka A, Poggiogalle E, et al. Effects of angiopoietin-like protein 3 deficiency on postprandial lipid and lipoprotein metabolism. *J Lipid Res*. 2016;57(6): 1097–1107.
43. Moraes-Vieira PM, Saghatelian A, Kahn BB. GLUT4 expression in adipocytes regulates De novo lipogenesis and levels of a novel class of lipids with antidiabetic and anti-inflammatory effects. *Diabetes*. 2016;65(7): 1808–1815.
44. Tramunt B, Smati S, Grandgeorge N, et al. Sex differences in metabolic regulation and diabetes susceptibility. *Diabetologia*. 2020;63(3):453–461.
45. Brutsaert EF, Shitole S, Biggs ML, et al. Relations of Postload and fasting glucose with incident cardiovascular disease and mortality late in life: the Cardiovascular Health Study. *J Gerontol A Biol Sci Med Sci*. 2016;71(3): 370–377.

Downloaded from https://academic.oup.com/aje/article/191/7/1235/6542790 by UC - San Francisco user on 05 October 2022