Published in final edited form as:

Osteoporos Int. 2021 September ; 32(9): 1745–1751. doi:10.1007/s00198-021-05897-4.

Serum Non-Esterified Fatty Acid Levels and Hip Fracture Risk in Older Adults:

The Cardiovascular Health Study

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Conflicts of interest/Competing interests: The authors declare that they have no conflict of interest.

Consent to participate: Included in the text under METHODS

Consent for publication: If accepted for publication the authors agree to publication.

Availability of data and material: Will supply if requested.

Code availability: Will supply if requested.

Declaration: All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Abstract

Purpose: Serum non-esterified fatty acids (NEFAs) are bioactive metabolic intermediates that can be taken up by bone tissue. Their associations with hip fracture risk have not been previously examined.

Methods: Thirty-five individual NEFAs in five classes (saturated [SFA], mono-un-saturated [MUFA], polyunsaturated n-6 and n-3 [PUFA], and *trans*-FA) were measured in Cardiovascular Health Study participants (n=2139, mean age 77.8 years) without known diabetes. The multivariable associations of NEFA levels with hip fracture risk were evaluated in Cox hazards models.

Results: We documented 303 incident hip fractures during 11.1 years of follow up. Among the five NEFA classes, total *trans* FA levels were positively associated with higher hip fracture risk (HR 1.17 [95% CI, 1.04, 1.31; p=0.01] per one standard deviation higher level). The SFA lignoceric acid (24:0) was positively associated with higher risk (HR 1.09 [1.04, 1.1]; p<0.001), while behenic (22:0) and docosatetraenoic (22:4 n6) acids were associated with lower risk (HR 0.76 [0.61, 0.94]; p=0.01; 0.84 [0.70, 1.00]; p=0.05; respectively).

Conclusion: Total plasma *trans* NEFA levels are related to hip fracture risk, suggesting an unrecognized benefit of their systematic removal from food. Novel associations of individual NEFAs with hip fracture risk require confirmation in other cohort studies.

Mini Abstract:

Among elderly participants from the Cardiovascular Health Study, we found that non-esterified *trans* fatty acid levels had a significant prospective association with hip fracture risk. Other non-esterified fatty acid classes were not associated with hip fracture risk.

Keywords

hip fracture; risk; NEFA; *trans* fatty acid; saturated fatty acids

INTRODUCTION

Plasma non-esterified fatty acids (NEFA) (also called free fatty acids (FFA)) are carboxylic acids with aliphatic carbon chains of varying lengths (12-24 carbons) and degrees of saturation (0-6 double bonds). They include saturated, mono- and poly-unsaturated, and *trans* fatty acids, the latter of which reflect consumption of hydrogenated oils, which have been restricted from foods owing to their putative adverse cardiovascular effects [1]. NEFA represent the output of the lipolysis of triacylglycerols (TG), stored in abdominal fat, that are transported to distant organs for energy utilization as needed by the body. At rest, 40% of FFA uptake occurs in the splanchnic bed and 20% in the legs. During exercise, NEFA delivery and output shifts from visceral organs to skeletal muscle, counterbalanced by increased lipolysis of fat stores [2]. In obesity, NEFA play a prominent role in the development of insulin resistance [3]. Enlarged visceral adipocytes overwhelm the portal

circulation with high levels of NEFA that cannot be completely oxidized, resulting in fat accumulation in non-adipose tissues (e.g., muscle, liver, and pancreatic beta-cells).

The lipolytic process inside adipose cells that releases NEFA into the circulation is associated with high levels of the cytokine TNF alpha [4]. Also, because NEFAs can induce inflammation, oxidative stress, and insulin resistance [5], all of which have been related to risk of hip fracture [6, 7], it is plausible that NEFA levels may be associated with fracture risk. In support of this possibility are two observations. First, type 2 diabetes – a condition of insulin resistance, inflammation, oxidative stress, and elevated NEFA levels – is associated with increased fracture risk, despite normal or increased bone mineral density levels [8]. Second, osteoblasts have receptors for NEFA [9, 10] and play an important role in peripheral NEFA clearance.

In the present study, we examine the prospective association of NEFA levels with hip fracture risk in a cohort of older adults enrolled in the Cardiovascular Health Study, an ongoing, population-based longitudinal study of older Americans. We hypothesized that hip fracture risk varies by levels of total, classes, and individual NEFA.

METHODS

Study Cohort:

The CHS is an ongoing longitudinal study of adults aged 65 years in four US communities drawn from Medicare lists [11]. In 1989-1990, 5201 participants were recruited, followed by an additional 687 African Americans in 1992-1993. All participants gave informed consent upon study entry. Institutional review board approval was received at all clinical sites.

From 1989-1990 to 1998-1999, participants were seen in clinic annually and had annual telephone contact between clinic visits. Following the 1998-1999 visit, participants continued to be contacted biennially to update hospitalizations, incident diagnoses, and medications. For the present study, the baseline year was the 1996-1997 visit when stored serum samples were used to measure NEFA levels. An oral glucose tolerance test (OGTT) was administered at this visit; hence participants with diabetes were excluded since OGTT were not performed on them. Participants with prior hip fractures were also excluded from these analyses.

NEFA Levels:

Fasting serum samples, collected at the 1996-1997 exam, were stored in the central laboratory at the University of Vermont. At the time of collection, the blood was allowed to clot for 60 minutes at room temperature. Serum was separated by centrifugation at $1100 \times g$ for 10 minutes at 4°C, and stored for subsequent analysis at -80 °C. At the time of analyses (at the Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA), serum was thawed at 4°C and lipids were extracted after addition of an internal standard (heptadecanoic acid, 20% w/v in chloroform) as previously described [12, 13]. The NEFA fraction was separated from the lipid extract using solid-phase chromatography (aminopropyl columns) [14]. The NEFA fraction was dried under nitrogen, saponified and methylated to form fatty acid methyl esters (FAMES) as previously

described [12, 15]. The FAMES were analyzed using an Autosystem XL gas chromatogram (Perkin Elmer, Boston, MA) equipped with a 100 m x 0.25 mm capillary column (HP INNOWax, Agilent Technologies, CA). Fatty acid peaks were identified by comparison with authenticated standards (NuCheck Prep, MN) and expressed as $\mu\text{mol/L}$. The inter-assay coefficients of variation were 0.5% to 4.3% for fatty acids present at concentrations >5 mol%, 1.8 to 7.1% for fatty acids present at concentrations between 1-5 mol%, and 2.8 to 11.1% for fatty acids present at concentrations <1 mol%. Individual NEFA were then summed to form classes and total NEFA. The correlation of total NEFA estimated by gas chromatography with a simultaneous measurement using an enzymatic method was 0.92.

Outcome:

The primary study outcome was incident hip fracture. Fracture data were obtained through patient-report and then confirmed through hospital medical records, including discharge summaries, gathered every 6 months from the 1996-1997 visit through June 30, 2015. Data were checked against Medicare claims data to identify any hospitalizations not reported by the participant. Hip fracture was defined using the International Classification of Diseases, Ninth Revision (ICD-9) code 820.xx. Pathological fractures (ICD-9 code 773.1x) and motor vehicle accidents (E810.xx-E825.xx) were excluded.

Covariates:

Analyses were adjusted for covariates taken from the baseline visit in 1996-1997. In Model 0 we adjusted for age, sex, race, and field center. In Model 1 we further adjusted for waist circumference (cm), hypertension, use of hypertension medication, a history of falling in the year before baseline, systolic and diastolic blood pressure, adjudicated forms of cardiovascular disease (coronary heart disease [CHD], stroke, heart failure, atrial fibrillation), estimated glomerular filtration rate based on cystatin C levels (eGFR_{cys}), smoking status (current, past, never), lipid levels, C-reactive protein levels (CRP), serum albumin, current alcohol use (none, 7 drinks per week, >7 drinks per week), gait speed (meters per second) [16], urine and serum albumin (mg/dl), physical activity level (none, moderate and high intensity [17]), and use of estrogen by women. The Gutt index of insulin sensitivity [18] and the Matsuda index [19] were calculated as previously reported.

Methods:

Baseline characteristics of the cohort were examined by quartiles of total NEFA. The inter-relationship of NEFA classes with one another (SFA, total MUFA, total PUFA n-6, PUFA n-3, total trans NEFA) was examined through Pearson correlations. To examine the associations of NEFA levels with incident hip fracture in as broad a manner as possible, we constructed two Cox proportional hazards models, each with 2 nested sets of covariates as listed above. In the first model, we estimated the associations between an incident hip fracture and a single exposure - an individual NEFA, a NEFA class, or total NEFA levels. In the second model, all 35 individual NEFA were assessed together. The association magnitudes were scaled per standard deviation (SD). For the analysis of individual NEFAs, we used multiple testing adjustment of p values by Benjamini-Yekutieli false discovery rate, valid for all dependence structures between tests [20].

We considered inflammation (as measured by CRP) and insulin sensitivity (as measured by Gutt and Matsuda indices) as possible intermediate factors between NEFA and hip fracture risk, but none of the three was associated with risk of hip fracture.

Analyses were done using R, 2019 [21].

RESULTS

Of the 4413 CHS participants at the baseline year, 2139 (48.5%) had NEFA levels measured. Those excluded typically did not attend the in-person clinic visit or had known diabetes. Those not included in the present study (2274; 51.5% of the total cohort) were older, more likely to be African American, had lower attained levels of education, were heavier, and had more cardiovascular comorbidity than those included in the study (Supplementary Table 1).

Supplementary Table 2 shows the distribution of total and individual NEFA. Total SFA had the highest levels, followed by total MUFA and PUFA n-6 levels. All the NEFA classes were correlated with one another (Supplementary Table 3). Correlation coefficients varied from moderate (*trans* vs omega 3, $R=0.58$) to high (total NEFA vs MUFA, $R = 0.97$).

Baseline factors and their distribution in the total cohort by quartiles of total NEFA levels are shown in Table 1. NEFAs were positively correlated with age, weight, HDL cholesterol level, systolic blood pressure, fasting and 2-hour glucose levels on an OGTT, fasting insulin levels, urine albumin, stroke and hypertension, and more use of estrogen by females. Total NEFA levels were also inversely associated with history of smoking, exercise, and with insulin sensitivity (Gutt index, Matsuda index).

Median follow up was 11.1 years (interquartile follow up, 6.32, 16.3 years) (from 1997 through June 2015). There were 303 incident hip fractures, for an incidence rate of 1.35 (95% CI, 1.005,1.82) fractures per 100 patient-years.

We first examined the association of total NEFA and its subclasses with risk of hip fracture (Table 2). Total NEFA and its subclasses tended to be associated with numerically higher risk of hip fracture, but these were not statistically significant in most cases. Only total *trans* NEFA (TFA) were significantly associated, with a ~17-18% higher risk per standard deviation increment. As seen in Table 2, many of the individual *trans* species were also associated with higher fracture risk.

In a second Cox model (Table 3), all 35 individual NEFA were considered together, i.e., adjusting the association of each NEFA with hip fracture risk for the associations of the other 34 species with hip fracture risk. In this model, the SFAs arachidic acid (20:0) and lignoceric acid (24:0) were associated with higher risk of hip fracture. Conversely, there were significant associations of the SFA behenoic acid (22:0) and the PUFA 6 docosatetraenoic acid (22:4) with lower risk of fracture; stearic acid (18:0) was associated with lower risk only in M1. Although total *trans* NEFA were associated with significantly higher risk, no single *trans* isomer met this threshold when included together, although elaidic acid (18: 1n-9t), the most common *trans* NEFA, was associated with significantly higher risk in M0 and nearly so (a non-significant 24% higher risk) in M1.

DISCUSSION

In this, the first study to our knowledge to examine individual NEFA levels with hip fracture risk, we identified two salient points. First, TFA as a whole and multiple individual species were associated with higher risk. Second, when individual NEFA levels were considered together and adjusted for each other's associations with fracture risk, we observed higher hip fracture risk in association with two specific but uncommon intermediate-chain SFA and lower risk with another SFA and a PUFA-6.

Unlike most other NEFA, TFA are derived exclusively from the diet. Most ingested TFA are generated during industrial processing of vegetable oils rich in PUFA through partial hydrogenation [22], though some comes from milk ingestion [23]. TFA inside cells alter membrane properties and lead to endoplasmic reticulum stress, reactive oxygen species, inflammation, and oxidative stress. Despite their relatively low ambient levels, in our study TFA were significantly associated with hip fracture risk, suggesting a potent effect by small amounts. Our findings are consistent with a retrospective analysis of the TRANSFAIR Study from 1999 [24]. In that dietary study, the amount of ingested TFA (grams/day) was significantly associated with hip fracture risk in men and women ($r^2 = 0.57$ men; 0.65 women: $p < 0.05$). In another study, incubation of osteoblasts from fish scales (similar to mammalian bone) with vaccenic acid (19:1t, n7) - the main TFA in dairy products - reduced collagen and osteocalcin production, suggestive of a detrimental effect on bone function [25].

The time of sample collection for this study was prior to the recognition of the detrimental effects of TFA and their subsequent removal from the US food supply. The levels of TFA reported here are therefore likely higher than what would be found today, enabling us to ascertain a harmful association that would be difficult to replicate in recently collected samples. Nonetheless, low levels of TFA continue to persist in the diet. A study using NHANES data showed only a 50-58% reduction in TFA levels in the US population from 2000 to 2010 [26]. Also, until recently, foods containing less than 0.5 grams of TFA were labelled as containing "zero" TFA [27]. Given the potency of several low serum level TFA (e.g., 16:1n7t, 18:1n7t) our findings have public health implications for hip fracture prevention. We note that higher food insecurity is associated with higher serum TFA levels [28], possibly making individuals of lower socio-economic status more susceptible to hip fracture.

We found no significant association of total SFA nor with palmitic acid (16:0), the most common single SFA, with hip fracture risk. On the other hand, lignoceric acid (24:0) and arachidic acid (20:0) had positive associations with hip fracture risk, albeit their serum levels were low. These findings are novel and should be considered exploratory, especially given the divergent associations with stearic and behenic acids.

This study did not directly measure biological mediators of the association of NEFA with hip fracture risk. However, the association of NEFA with hip fracture risk did not appear to be mediated through inflammation, as measured by CRP levels, nor with insulin sensitivity, as measured by the Gutt and Matsuda indices. Hence, the mechanisms that

underlie the associations observed here are necessarily speculative. We note that bone cells have receptors for NEFA [9, 10] so that a direct effect of NEFA on hip fracture risk is one possible mechanism of association.

Strengths of this study include a well characterized cohort and adjustment of risk estimates for numerous factors associated with hip fracture risk. We documented over 300 hip fractures, providing for both precision and the ability to control simultaneously for covariates and the full spectrum of NEFA species. Assays for NEFA levels were conducted in a dedicated research lipid laboratory with high sensitivity. Finally, this investigation is novel and has not been performed previously to our knowledge. Limitations of this study include exclusion of adults with known glucose disorders, thereby limiting the generalizability of our findings. It is possible that the higher NEFA levels present with diabetes would have had an even stronger association with hip fracture risk and help to explain the increased risk of osteoporotic fractures in people with diabetes. Second, we did not have contemporaneous measures of bone density; hence, we could not assess whether NEFA influence hip fracture by affecting bone density, bone quality, or some other factor related to bone remodeling. Third, we measured NEFA from only a single visit, precluding us from examining changes in levels over time.

In conclusion, many studies have examined dietary intake of fats or phospholipid measurements with hip fracture risk but NEFA have not received comparable attention. Our results suggest that further attention to NEFA species may be warranted, particularly given the consistently detrimental effects of TFA, and the robust associations of two low-frequency SFA with higher risk. Additional studies are needed to explore biological mechanisms by which individual NEFA influence this risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding:

This research was supported by contracts HHSN268201200036C, HHSN268200800007C, HHSN268201800001C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and grants U01HL080295 and U01HL130114 from the National Heart, Lung, and Blood Institute (NHLBI), with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided by R01AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org.

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Table 1:

Baseline characteristics of the sub-cohort with free fatty acid measurements in the Cardiovascular Health Study based on quartiles of total NEFA. P values are based on linear trend tests across quartiles for continuous variables and Chi squared tests for categorical variables.

	Q1 N=535	Q2 N=535	Q3 N=534	Q4 N=535	P Value
Demographic					
Age (years)	77.29 ± 3.99	77.93 ± 4.54	77.99 ± 4.57	78.25 ± 4.83	<0.01
Sex (Male) (%)	60.9	47.1	33.1	17.6	<0.001
BMI (kg/m ²)	26.18 ± 3.68	26.36 ± 4.17	26.22 ± 4.54	26.88 ± 5.04	<0.001
Waist circumference (cm)	95.82 ± 11.16	95.68 ± 11.73	97.80 ± 12.73	95.96 ± 14.59	0.30
Systolic BP (mmHg)	132.80 ± 20.35	136.32 ± 20.22	137.31 ± 19.85	139.84 ± 20.95	<0.001
Diastolic BP (mmHg)	69.95 ± 11.16	69.10 ± 11.01	70.69 ± 9.95	69.98 ± 11.89	0.43
Race (Black) (%)	15.0	12.9	15.2	13.3	0.62
Educ >12 years (%)	57.5	51.6	46.7	46.9	<0.001
Smoking status (%)					<0.001
Current	10.4	8.8	7.2	5.7	
Former	50.0	41.7	43.5	36.8	
Never	39.6	49.5	49.3	57.5	
Physical activity (%)					<0.001
0 (least active)	17.9	19.1	23.5	29.5	
1 (moderately active)	47.8	51.4	54.7	48.8	
2 (most active)	34.3	29.5	21.8	21.7	
Gait speed (meter/second)	0.98 ± 0.24	0.93 ± 0.25	0.90 ± 0.24	0.86 ± 0.26	<0.001
Laboratory					
Cholesterol (mg/dl)	193.37 ± 38.63	201.46 ± 39.65	201.67 ± 37.68	211.11 ± 36.58	<0.001
Fasting glucose (mg/dl)	96.38 ± 13.25	98.03 ± 16.21	98.71 ± 13.00	98.93 ± 16.11	0.003
2-hour glucose (mg/dl)	122.00 ± 44.49	138.31 ± 50.17	153.23 ± 48.96	164.64 ± 57.44	<0.001
Fasting insulin (mIU/L)	13.33 ± 7.19	13.38 ± 5.12	13.82 ± 5.56	13.99 ± 6.38	0.04
2-hour insulin (mIU/L)	65.48 ± 46.08	78.02 ± 52.72	91.09 ± 65.70	93.66 ± 73.16	<0.001
Log ₂ (CRP) (mg/L)	1.03 ± 1.56	1.20 ± 1.61	1.38 ± 1.58	1.29 ± 1.53	0.002
Urine albumin (mg/dl)	61.02 ± 349.34	37.17 ± 150.54	37.83 ± 189.7	51.33 ± 267.58	0.57
Serum albumin (mg/dl)	3.78 ± 0.30	3.82 ± 0.28	3.81 ± 0.28	3.90 ± 0.30	<0.001
Log ₂ (CRP)	1.01 ± 1.57	1.27 ± 1.58	1.35 ± 1.57	1.27 ± 1.56	0.005
eGFR _{cys} (ml/min/1.73 meter ²)	70.51 ± 16.96	71.59 ± 20.66	71.85 ± 18.06	72.75 ± 19.41	0.06
Insulin Sensitivity					
Gutt index	72.43 ± 24.95	63.52 ± 21.21	56.71 ± 19.65	54.56 ± 20.46	<0.001
Matsuda index	4.39 ± 2.39	3.58 ± 2.1	3.21 ± 1.87	2.94 ± 1.83	<0.001
Medical History (%)					

	Q1 N=535	Q2 N=535	Q3 N=534	Q4 N=535	P Value
CHD	25.8	21.3	20.4	17.9	0.02
Stroke	4.1	6.2	5.1	5.6	0.48
Hypertension	53.5	57.4	62.6	66.1	<0.001
Hypertension medication use	50.8	51.8	55.2	54.7	0.39
Heart failure	5.0	7.9	5.6	6.9	0.23
Atrial fibrillation	4.1	4.3	2.8	5.6	0.32
Fall year before baseline	14.3	14.7	17.8	20.8	0.01
Estrogen use (female)	18.7	16.3	18.2	22.4	0.20

Table 2:

Cox regression for incident hip fracture, per one standard deviation of each NEFA class. Model 0 (**M0**) is adjusted for age, gender, black race, and clinic of participation. Model1 (**M1**) is further adjusted for highest attained level of education, smoking status (current, never, former), alcohol use (none, <7 drinks per week, >7 drinks per week), weight, height, cholesterol, HTN, HTN medications, SBP, DBP, previous MI and/or stroke, heart failure, serum albumin, eGFR based on cystatin C level, degree of physical activity (none, moderate, and strenuous).

		M0				M1		
		HR	95% CI	P Value		HR	95% CI	P Value
Total NEFA		1.06	0.94, 1.19	0.38		1.08	0.95, 1.24	0.22
Total SFA		1.02	0.91, 1.15	0.76		1.05	0.92, 1.91	0.47
Total MUFA		1.05	0.93, 1.19	0.44		1.09	0.95, 1.24	0.23
Total PUFA 6		1.10	0.98, 1.23	0.13		1.10	0.97, 1.24	0.14
Total PUFA 3		1.05	0.94, 1.18	0.40		1.07	0.95, 1.21	0.27
TFA		1.18	1.06, 1.33	0.004		1.17	1.04, 1.31	0.01

Table 3:

Cox regression for incident hip fracture. All NEFA are in a single model. **Model 0 (M0)** is adjusted for age, gender, black race, and clinic of participation; **Model 1 (M1)** is further adjusted for highest attained education level, smoking status (current, never, former), alcohol use (none, <7 drinks per week, > 7 drinks per week), weight, height, cholesterol, HTN, HTN medications, SBP, DBP, previous MI and/or stroke, heart failure, serum albumin, eGFR based on cystatin C level, degree of physical activity (none, moderate, and strenuous). Results are reported per one standard deviation increase of the NEFA.

	M0				M1		
	HR	95% CI	P Value		HR	95% CI	P Value
SFA							
12	1.05	0.94, 1.18	0.39		1.05	0.94, 1.18	0.40
14	1.02	0.66, 1.59	0.93		0.98	0.62, 1.55	0.94
15	1.07	0.76, 1.51	0.68		1.07	0.76, 1.51	0.69
16	0.98	0.64, 1.48	0.91		1.08	0.69, 1.67	0.74
18	0.79	0.59, 1.06	0.11		0.74	0.55, 1.00	0.047
20	1.34	1.11, 1.61	0.002		1.39	1.14, 1.68	<0.001
22	0.77	0.63, 0.94	0.01		0.76	0.61, 0.94	0.009
24	1.09	1.04, 1.14	<0.001		1.09	1.04, 1.15	<0.001
MUFA							
14 ln 5	1.18	0.80, 1.73	0.40		1.20	0.80, 1.80	0.39
16 ln 9	1.12	0.79, 1.57	0.54		1.16	0.83, 1.63	0.38
16 ln 7	0.83	0.50, 1.38	0.47		0.75	0.43, 1.31	0.31
18 ln 9	0.72	0.35, 1.47	0.37		0.85	0.41, 1.78	0.67
18 ln 7	1.03	0.59, 1.78	0.93		0.98	0.55, 1.76	0.95
20 ln 9	1.20	0.85, 1.69	0.30		1.17	0.82, 1.68	0.39
22 ln 9	1.05	0.92, 1.12	0.43		1.06	0.93, 1.21	0.37
24 ln 9	1.05	0.97, 1.14	0.22		1.09	0.97, 1.21	0.14
PUFA 6							
18 2 n6	1.29	0.84, 1.97	0.25		1.08	0.69, 1.66	0.75
18 3 n6	1.07	0.90, 1.27	0.44		1.09	0.92, 1.29	0.32
20 2 n6	1.05	0.90, 1.21	0.57		1.08	0.92, 1.26	0.36
20 3 n6	1.02	0.78, 1.34	0.88		1.09	0.83, 1.44	0.53
20 4 n6	1.03	0.84, 1.28	0.76		0.97	0.78, 1.21	0.77
22 4 n6	0.85	0.72, 1.00	0.06		0.84	0.70, 1.00	0.049
22 5 n6	1.00	0.84, 1.19	0.96		1.00	0.83, 1.21	1.00
PUFA 3							
18 3 n3	1.01	0.77, 1.33	0.92		1.04	0.79, 1.38	0.77
18 4 n3	1.00	0.86, 1.16	0.96		1.01	0.87, 1.18	0.86

	M0				M1		
	HR	95% CI	P Value		HR	95% CI	P Value
20 5 n3	0.99	0.80, 1.24	0.95		0.99	0.79, 1.24	0.91
22 5 n3	0.74	0.54, 1.00	0.049		0.75	0.55, 1.04	0.08
22 6 n3	1.13	0.92, 1.38	0.23		1.13	0.91, 1.41	0.28
TFA							
16 ln 9t	0.82	0.59, 1.13	0.22		0.88	0.63, 1.24	0.48
16 ln 7t	0.92	0.66, 1.29	0.64		0.94	0.67, 1.32	0.72
18 ln 101 2t	1.40	0.99, 1.99	0.06		1.36	0.95, 1.93	0.09
18 ln 9t	1.29	0.93, 1.79	0.13		1.24	0.89, 1.73	0.21
18 ln 7t	0.99	0.71, 1.38	0.94		0.94	0.67, 1.33	0.73
18 2t	1.06	0.96, 1.17	0.27		1.05	0.94, 1.17	0.36
18 clat	0.84	0.71, 0.99	0.042		0.85	0.71, 1.01	0.07