

**Supplement Table 1:** Markers of systemic inflammation and oxidative stress by participant hepatic steatosis status

	<b>Hepatic steatosis</b> (n = 425)	<b>No hepatic steatosis</b> (n = 2057)	<b>Overall</b> (n = 2482)
Cluster of differentiation 40 (CD40) ligand (ng/mL)	3.0±4.3 (n = 186)	3.7±5.2 (n = 803)	3.6±5.1 (n = 989)
Fibrinogen (mg/dL)	390±67 (n = 186)	375±71 (n = 802)	378±71 (n = 988)
High-sensitivity C-reactive protein (hs-CRP, mg/L)	4.8±5.4 (n = 425)	2.7±5.0 (n = 2055)	3.1±5.2 (n = 2480)
Intercellular adhesion molecule 1 (ICAM1, ng/mL)	282±95 (n = 424)	250±68 (n = 2054)	255±74 (n = 2478)
Interleukin 6 (IL6, pg/mL)	3.4±3.9 (n = 424)	2.5±4.1 (n = 2055)	2.7±4.1 (n = 2479)
Lipoprotein-phospholipase A2 (Lp-PLA2) activity (nmol/mL/min)	164±39 (n = 425)	160±37 (n = 2053)	161±38 (n = 2478)
Lipoprotein-phospholipase A2 (Lp-PLA2) mass (ng/mL)	261±79 (n = 425)	261±73 (n = 2053)	261±74 (n = 2478)
Monocyte chemoattractant protein 1 (MCP1, pg/mL)	361±121 (n = 417)	345±135 (n = 2015)	347±133 (n = 2432)
Myeloperoxidase (MPO, ng/mL)	49.0±35.9 (n = 182)	49.3±31.6 (n = 776)	49.3±32.4 (n = 958)
Osteoprotegerin (pmol/L)	5.1±2.0 (n = 425)	4.8±1.6 (n = 2054)	4.8±1.7 (n = 2479)
P-selectin (ng/mL)	50.1±20.7 (n = 425)	44.4±17.6 (n = 2054)	45.4±18.2 (n = 2479)
TNF receptor-2 (TNFR2, pg/mL)	2357±811 (n = 417)	2493±12942 (n = 2041)	2470±11798 (n = 2458)
TNF-alpha (TNF- $\alpha$ , pg/mL)	1.5±1.8 (n = 138)	1.4±1.3 (n = 598)	1.4±1.4 (n = 736)
Urinary isoprostanes (pg/mL)	1632±1519 (n = 388)	1169±1215 (n = 1900)	1247±1283 (n = 2288)

Mean±standard deviation for continuous variables or n (%) for categorical variables  
Hepatic steatosis defined by Liver:Phantom Ratio  $\leq$  0.33

**Supplement Table 2:** Age- and Sex-adjusted correlation coefficients between liver fat (by Liver:Phantom Ratio) and markers of inflammation or oxidative stress

<b>Markers of inflammation or oxidative stress</b>	<b>Correlation coefficient</b>	<b>P-value</b>
Cluster of differentiation 40 (CD40) ligand	0.02 (n=989)	0.59
Fibrinogen	<b>0.11 (n=988)</b>	<b>&lt;0.001</b>
High-sensitivity C-reactive protein (hs-CRP)	<b>0.27 (n=2480)</b>	<b>&lt;0.001</b>
Intercellular adhesion molecule 1 (ICAM1)	<b>0.18 (n=2478)</b>	<b>&lt;0.001</b>
Interleukin 6 (IL6)	<b>0.18 (n=2479)</b>	<b>&lt;0.001</b>
Lipoprotein-phospholipase A2 (Lp-PLA2) activity	<b>0.04 (n=2478)</b>	<b>0.03</b>
Lipoprotein-phospholipase A2 (Lp-PLA2) mass	-0.02 (n=2478)	0.28
Monocyte chemoattractant protein 1 (MCP1)	<b>0.05 (n=2432)</b>	<b>0.02</b>
Myeloperoxidase (MPO)	-0.01 (n=958)	0.83
Osteoprotegerin	<b>0.05 (n=2479)</b>	<b>0.01</b>
P-selectin	<b>0.12 (n=2479)</b>	<b>&lt;0.001</b>
TNF receptor-2 (TNFR2)	<b>0.07 (n=2458)</b>	<b>&lt;0.001</b>
TNF-alpha (TNF- $\alpha$ )	0.02 (n=736)	0.55
Urinary isoprostanes	<b>0.14 (n=2288)</b>	<b>&lt;0.001</b>

**Supplement Table 3: Sensitivity analyses-** Assessment of interaction with sex and obesity among parameters with significant association with Liver:Phantom Ratio (LPR)

Markers of inflammation with a significant interaction with sex

Markers of inflammation or oxidative stress	Model 3 All participants		Model 3 Women only		Model 3 Men only	
	$\beta$ (95% CI)	<i>P</i> -value	$\beta$ (95% CI)	<i>P</i> -value	$\beta$ (95% CI)	<i>P</i> -value
Urinary isoprostanes	<b>0.87 (0.43, 1.31)</b>	<b>&lt;0.001</b>	<b>1.22 (0.51, 1.94)</b>	<b>&lt;0.001</b>	<b>0.58 (0.05, 1.12)</b>	<b>0.033</b>

Markers of inflammation with a significant interaction with obesity (BMI > 30 kg/m<sup>2</sup>)

Markers of inflammation or oxidative stress	Model 3 All participants		Model 3 Obese only		Model 3 Non-obese only	
	$\beta$ (95% CI)	<i>P</i> -value	$\beta$ (95% CI)	<i>P</i> -value	$\beta$ (95% CI)	<i>P</i> -value
High-sensitivity C-reactive protein (hs-CRP)	<b>1.23 (0.81, 1.65)</b>	<b>&lt;0.001</b>	<b>1.93 (1.33, 2.54)</b>	<b>&lt;0.001</b>	<b>0.67 (0.12, 1.23)</b>	<b>0.02</b>
Interleukin 6 (IL6)	<b>0.50 (0.23, 0.77)</b>	<b>&lt;0.001</b>	<b>0.80 (0.42,1.19)</b>	<b>&lt;0.001</b>	0.18 (-0.18, 0.54)	0.34

Estimates in table give change in log<sub>e</sub> biomarker for a 1 standard deviation increase in liver fat (measured via the liver phantom ratio) with (95% CI) and p-value

“Model 3” Adjusts for age, sex, smoking status, alcohol consumption, regular aspirin use, hypertension, lipid treatment, total/high density lipoprotein cholesterol ratio, triglycerides, diabetes, prevalent cardiovascular disease, and BMI (Body Mass Index)

Cardiovascular disease: participant-reported history of coronary heart disease, stroke, heart failure, or intermittent claudication adjudicated by review of medical records

Regular Aspirin use: chronic use of three or more doses of aspirin per week

Hypertension: systolic blood pressure  $\geq$  140 mmHg, diastolic blood pressure  $\geq$  90 mmHg, or use of any anti-hypertensive medication

Diabetes: fasting blood glucose  $\geq$  126 mg/dL or treatment with an anti-hyperglycemic medication

Lipid-lowering therapy: treatment with any medication for “high-cholesterol” or “high-triglycerides”

**Supplement Table 4:** Sensitivity analysis: Comparisons of multivariable linear regression analysis results after exclusion of participants with diabetes or cardiovascular disease

Markers of inflammation or oxidative stress	Model 3		Model 3 <i>excluding subjects with diabetes or cardiovascular disease</i>	
	$\beta$ (95% CI)	P-value	$\beta$ (95% CI)	P-value
Cluster of differentiation 40 (CD40) ligand	0.47 (-0.34, 1.27)	0.26	0.13 (-0.85, 1.11)	0.80
Fibrinogen	0.02 (-0.08, 0.13)	0.67	-0.02 (-0.15, 0.11)	0.75
High-sensitivity C-reactive protein (hs-CRP)	<b>1.23 (0.81, 1.65)</b>	<b>&lt;0.001</b>	<b>1.30 (0.84, 1.77)</b>	<b>&lt;0.001</b>
Intercellular adhesion molecule 1 (ICAM1)	<b>0.30 (0.20, 0.40)</b>	<b>&lt;0.001</b>	<b>0.33 (0.22, 0.44)</b>	<b>&lt;0.001</b>
Interleukin 6 (IL6)	<b>0.50 (0.23, 0.77)</b>	<b>&lt;0.001</b>	<b>0.50 (0.21, 0.80)</b>	<b>&lt;0.001</b>
Lipoprotein-phospholipase A2 (Lp-PLA2) activity	0.02 (-0.05, 0.09)	0.62	0.03 (-0.05, 0.11)	0.50
Lipoprotein-phospholipase A2 (Lp-PLA2) mass	-0.09 (-0.19, 0.02)	0.11	-0.07 (-0.18, 0.05)	0.25
Monocyte chemoattractant protein 1 (MCP1)	0.07 (-0.08, 0.22)	0.34	0.01 (-0.15, 0.17)	0.88
Myeloperoxidase (MPO)	-0.13 (-0.51, 0.25)	0.50	-0.14 (-0.59, 0.31)	0.54
Osteoprotegerin	0.13 (-0.01, 0.26)	0.06	0.09 (-0.06, 0.24)	0.25
P-selectin	<b>0.20 (0.04, 0.36)</b>	<b>0.01</b>	<b>0.23 (0.05, 0.41)</b>	<b>0.01</b>
TNF receptor-2 (TNFR2)	0.02 (-0.10, 0.14)	0.76	0.06 (-0.07, 0.19)	0.34
TNF-alpha (TNF- $\alpha$ )	-0.21 (-0.57, 0.15)	0.25	0.00 (-0.43, 0.43)	0.99
Urinary isoprostanes	<b>0.87 (0.43, 1.31)</b>	<b>&lt;0.001</b>	<b>0.68 (0.19, 1.17)</b>	<b>0.007</b>

Estimates in table give change in log<sub>e</sub> biomarker for a 1 standard deviation increase in liver fat (measured via the liver phantom ratio) with (95% CI) and p-value

Model 3 adjusts for age, sex, smoking status, alcohol consumption, regular aspirin use, hypertension, lipid treatment, total/high density lipoprotein cholesterol ratio, triglycerides, diabetes, prevalent cardiovascular disease, and Body-Mass Index.

Cardiovascular disease: participant-reported history of coronary heart disease, stroke, heart failure, or intermittent claudication adjudicated by review of medical records

Regular Aspirin use: chronic use of three or more doses of aspirin per week

Hypertension: systolic blood pressure > 140 mmHg, diastolic blood pressure > 90 mmHg, or use of any anti-hypertensive medication

Diabetes: fasting blood glucose > 126 mg/dL or treatment with an anti-hyperglycemic medication

Lipid-lowering therapy: treatment with any medication for "high-cholesterol" or "high-triglycerides"

**Supplement Table 5:** Sensitivity analysis: Comparisons of multivariable linear regression analysis results after exclusion of participants with diabetes or cardiovascular disease

Markers of inflammation or oxidative stress	Model 3		Model 3 <i>excluding subjects with diabetes or cardiovascular disease</i>	
	$\beta$ (95% CI)	P-value	$\beta$ (95% CI)	P-value
Cluster of differentiation 40 (CD40) ligand	0.47 (-0.34, 1.27)	0.26	0.13 (-0.85, 1.11)	0.80
Fibrinogen	0.02 (-0.08, 0.13)	0.67	-0.02 (-0.15, 0.11)	0.75
High-sensitivity C-reactive protein (hs-CRP)	<b>1.23 (0.81, 1.65)</b>	<b>&lt;0.001</b>	<b>1.30 (0.84, 1.77)</b>	<b>&lt;0.001</b>
Intercellular adhesion molecule 1 (ICAM1)	<b>0.30 (0.20, 0.40)</b>	<b>&lt;0.001</b>	<b>0.33 (0.22, 0.44)</b>	<b>&lt;0.001</b>
Interleukin 6 (IL6)	<b>0.50 (0.23, 0.77)</b>	<b>&lt;0.001</b>	<b>0.50 (0.21, 0.80)</b>	<b>&lt;0.001</b>
Lipoprotein-phospholipase A2 (Lp-PLA2) activity	0.02 (-0.05, 0.09)	0.62	0.03 (-0.05, 0.11)	0.50
Lipoprotein-phospholipase A2 (Lp-PLA2) mass	-0.09 (-0.19, 0.02)	0.11	-0.07 (-0.18, 0.05)	0.25
Monocyte chemoattractant protein 1 (MCP-1)	0.07 (-0.08, 0.22)	0.34	0.01 (-0.15, 0.17)	0.88
Myeloperoxidase (MPO)	-0.13 (-0.51, 0.25)	0.50	-0.14 (-0.59, 0.31)	0.54
Osteoprotegerin	0.13 (-0.01, 0.26)	0.06	0.09 (-0.06, 0.24)	0.25
P-selectin	<b>0.20 (0.04, 0.36)</b>	<b>0.01</b>	<b>0.23 (0.05, 0.41)</b>	<b>0.01</b>
TNF receptor-2 (TNFR2)	0.02 (-0.10, 0.14)	0.76	0.06 (-0.07, 0.19)	0.34
TNF-alpha (TNF- $\alpha$ )	-0.21 (-0.57, 0.15)	0.25	0.00 (-0.43, 0.43)	0.99
Urinary isoprostanes	<b>0.87 (0.43, 1.31)</b>	<b>&lt;0.001</b>	<b>0.68 (0.19, 1.17)</b>	<b>0.007</b>

Estimates in table give change in log<sub>e</sub> biomarker for a 1 standard deviation increase in liver fat (measured via the liver phantom ratio) with (95% CI) and p-value

Model 3 adjusts for age, sex, smoking status, alcohol consumption, regular aspirin use, hypertension, lipid treatment, total/high density lipoprotein cholesterol ratio, triglycerides, diabetes, prevalent cardiovascular disease, and Body-Mass Index.

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Hypertension: systolic blood pressure > 140 mmHg, diastolic blood pressure > 90 mmHg, or use of any anti-hypertensive medication

Diabetes: fasting blood glucose > 126 mg/dL or treatment with an anti-hyperglycemic medication

Lipid-lowering therapy: treatment with any medication for "high-cholesterol" or "high-triglycerides"

**Supplement Table 6:** Difference in mean natural log-transformed markers of inflammation and oxidative stress among participants with hepatic steatosis with and without elevated ALT

Markers of inflammation or oxidative stress	Difference in mean log-transformed inflammatory markers between: those with hepatic steatosis with elevated ALT (n = 245) vs. those with hepatic steatosis without elevated ALT (n = 180)	P-value
Cluster of differentiation 40 (CD40) ligand	-0.17 (-0.53, 0.19)	0.36
Fibrinogen	0.00 (-0.05, 0.05)	0.90
High-sensitivity C-reactive protein (hs-CRP)	-0.03 (-0.25, 0.18)	0.76
Intercellular adhesion molecule 1 (ICAM1)	<b>0.12 (0.07, 0.16)</b>	<b>&lt;0.001</b>
Interleukin 6 (IL6)	-0.00 (-0.13, 0.12)	0.98
Lipoprotein-phospholipase A2 (Lp-PLA2) activity	<b>0.08 (0.04, 0.12)</b>	<b>&lt;0.001</b>
Lipoprotein-phospholipase A2 (Lp-PLA2) mass	0.01 (-0.04, 0.06)	0.61
Monocyte chemoattractant protein 1 (MCP-1)	0.07 (-0.00, 0.13)	0.05
Myeloperoxidase (MPO)	0.09 (-0.08, 0.25)	0.30
Osteoprotegerin	-0.05 (-0.11, 0.02)	0.14
P-selectin	<b>0.15 (0.07, 0.22)</b>	<b>&lt;0.001</b>
TNF receptor-2 (TNFR2)	<b>0.11 (0.05, 0.16)</b>	<b>&lt;0.001</b>
TNF-alpha (TNF- $\alpha$ )	0.15 (-0.01, 0.32)	0.07
Urinary isoprostanes	-0.01 (-0.21, 0.20)	0.96

ALT (Alanine aminotransferase)

Adjusted for age, sex, smoking status, alcohol consumption, and regular aspirin use based on model using pooled estimate of standard deviation from an ANCOVA model including all participants

## **Supplemental materials: Methods**

### *Covariate measurement and definitions*

For the second-generation participants, covariates were assessed at the 7<sup>th</sup> exam cycle (1998-2001) and for the third-generation participants, covariates were assessed at the 1<sup>st</sup> examination (2002-2005) which corresponded to the timing of the computed tomography scan.

### *Measurement of markers of systemic inflammation and oxidative*

Only a subset of the entire cohort was enrolled for certain biomarker measures (i.e. Cluster of differentiation 40 (CD40) ligand, fibrinogen, myeloperoxidase, and tumor necrosis factor-alpha (TNF-  $\alpha$ )). Thus, the sample size for each biomarker varied based upon the number of available measurements. Biomarkers were measured on fasting morning samples as previously described.<sup>1,2</sup> The intra-assay coefficients of variation for the biomarkers were < 10%.<sup>2,3</sup>

See Pou<sup>1</sup> et al. Supplemental materials:

Samples were stored at -80°C and thawed at the time of analysis (with exception of urine). Serum CRP serum was measured by high-sensitivity assay [(Dade Behring BN100 nephelometer; mean intra-assay CV 3.2%]. Fibrinogen was measured in duplicate from citrated plasma using Clauss method (Diagnostica Stago Reagents, CV 2.1%). Other markers were measured in duplicate by enzyme-linked immunosorbent assay commercially available kits [R&D Systems: intercellular adhesion molecule-1 (ICAM-1), interleukin-6, monocyte chemoattractant protein-1 (MCP-1), P-selectin, tumor necrosis factor receptor 2 (TNFR2), high-sensitivity TNF-alpha (TNF $\alpha$ ); Bender MedSystems: CD40 ligand; GlaxoSmithKline: lipoprotein associated phospholipase A2 (Lp-PLA2) activity and

mass; OXIS: myeloperoxidase; ALPCO Diagnostics: osteoprotegerin)]. Mean intra-assay CVs were as follows: plasma specimens: CD40 ligand 4.4%, fibrinogen 1.1%, Lp-PLA2 activity 7.0%, Lp-PLA2 mass 5%, osteoprotegerin 3.7%, P-selectin 3.0%, TNF $\alpha$  6.6%, TNFR2 2.2%; serum specimens: ICAM-1 3.7%, interleukin-6 3.1%, MCP1 3.8%, myeloperoxidase 3.0%, osteoprotegerin 3.7%. Isoprostane (8epi-PGF2 $\alpha$ ) production was measured in duplicate from urine samples using a commercially available enzyme-linked immunosorbent assay (Cayman, Ann Arbor, MI; CV 9.6 $\pm$  6.8), and indexed to urinary creatinine concentrations (Abbot Spectrum CCX; CV 2-4%), expressed as ng/mmol, as previously described.<sup>3</sup>

### *References*

1. Pou KM, Massaro JM, Hoffmann U, et al. Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: The Framingham Heart Study. *Circulation* 2007;116:1234-1241.
2. Jefferson AL, Massaro JM, Wolf PA, et al. Inflammatory biomarkers are associated with total brain volume. *Neurology* 2007;68:1032-1038.
3. Keaney JF, Larson MG, Vasan RS, et al. Obesity and systemic oxidative stress: Clinical correlates of oxidative stress in the Framingham study. *Arterioscler Thromb Vasc Biol* 2003;23:434-439.



	CD40	hs-CRP	Fibrinogen	ICAM <sub>1</sub>	IL6	Urinary Isoprostanes	Lp-PLA2 mass	MCP <sub>1</sub>	MPO	Osteoprotegerin	P-selectin	TNF- $\alpha$	TNFR-2	Lp-PLA2 activity
CD40		0.00	-0.01	0.07	-0.06	-0.05	-0.10	-0.04	-0.01	-0.07	-0.01	0.07	0.02	-0.02
hs-CRP	0.00		0.47	0.22	0.48	0.15	0.05	0.05	0.07	0.06	0.11	0.10	0.21	-0.09
Fibrinogen	-0.01	0.47		0.18	0.30	0.04	-0.01	0.08	0.06	0.03	0.13	0.12	0.14	-0.07
ICAM1	0.07	0.22	0.18		0.21	0.10	0.10	0.12	0.07	0.10	0.22	0.22	0.33	0.20
IL6	-0.06	0.48	0.30	0.21		0.17	0.11	0.05	0.10	0.05	0.05	0.24	0.17	-0.02
Urinary Isoprostanes	-0.05	0.15	0.04	0.10	0.17		0.06	0.07	0.13	0.04	0.05	0.01	0.00	0.06
Lp-PLA2 mass	-0.10	0.05	-0.01	0.10	0.11	0.06		-0.01	0.05	0.02	0.01	0.08	0.05	0.36
MCP1	-0.04	0.05	0.08	0.12	0.05	0.07	-0.01		0.18	0.02	0.17	0.10	0.19	0.11
MPO	-0.01	0.07	0.06	0.07	0.10	0.13	0.05	0.18		-0.01	0.10	0.06	0.07	0.08
Osteoprotegerin	-0.07	0.06	0.03	0.10	0.05	0.04	0.02	0.02	-0.01		-0.01	0.08	0.08	-0.06
P-selectin	-0.01	0.11	0.13	0.22	0.05	0.05	0.01	0.17	0.10	-0.01		0.13	0.22	0.21
TNF- $\alpha$	0.07	0.10	0.12	0.22	0.24	0.01	0.08	0.10	0.06	0.08	0.13		0.31	0.14
TNFR2	0.02	0.21	0.14	0.33	0.17	0.00	0.05	0.19	0.07	0.08	0.22	0.31		0.20
Lp-PLA2 activity	-0.02	-0.09	-0.07	0.20	-0.02	0.06	0.36	0.11	0.08	-0.06	0.21	0.14	0.20	

	hs-CRP	ICAM 1	IL6	Urinary Isoprostanes	P-selectin
hs-CRP		0.22	0.48	0.15	0.11
ICAM1	0.22		0.21	0.10	0.22
IL6	0.48	0.21		0.17	0.05
Urinary Isoprostanes	0.15	0.10	0.17		0.05
P-selectin	0.11	0.22	0.05	0.05	

Correlation coefficients for the markers with significant associations with hepatic steatosis in Model 3.