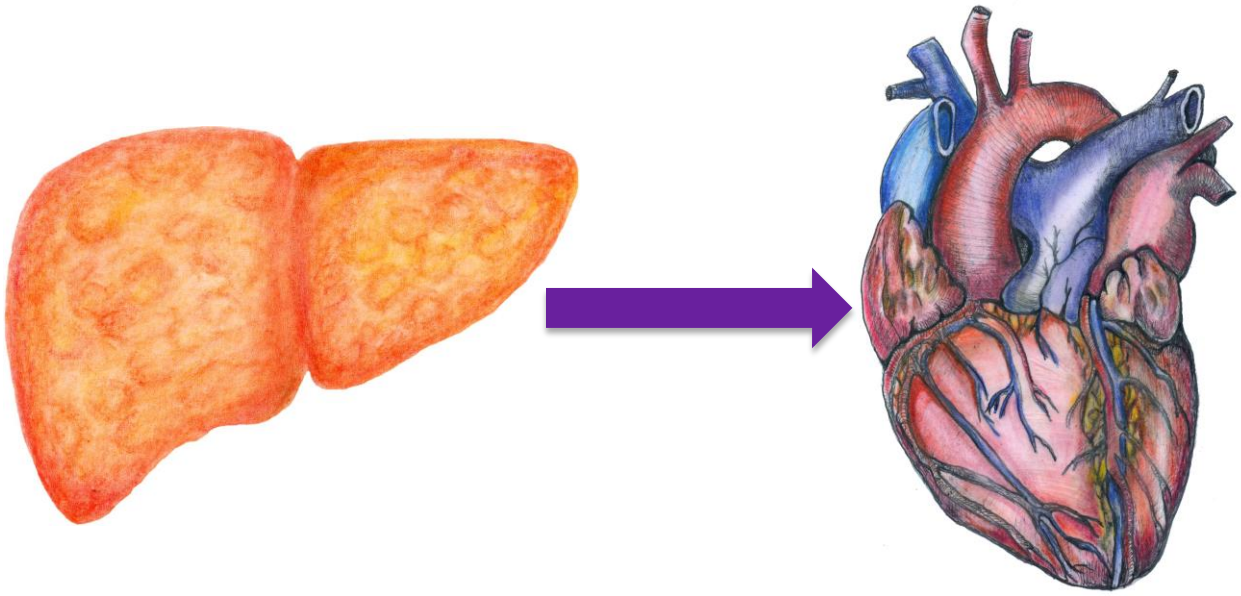

NAFLD and cardiovascular disease: What is the connection?

Paris NASH symposium 2016

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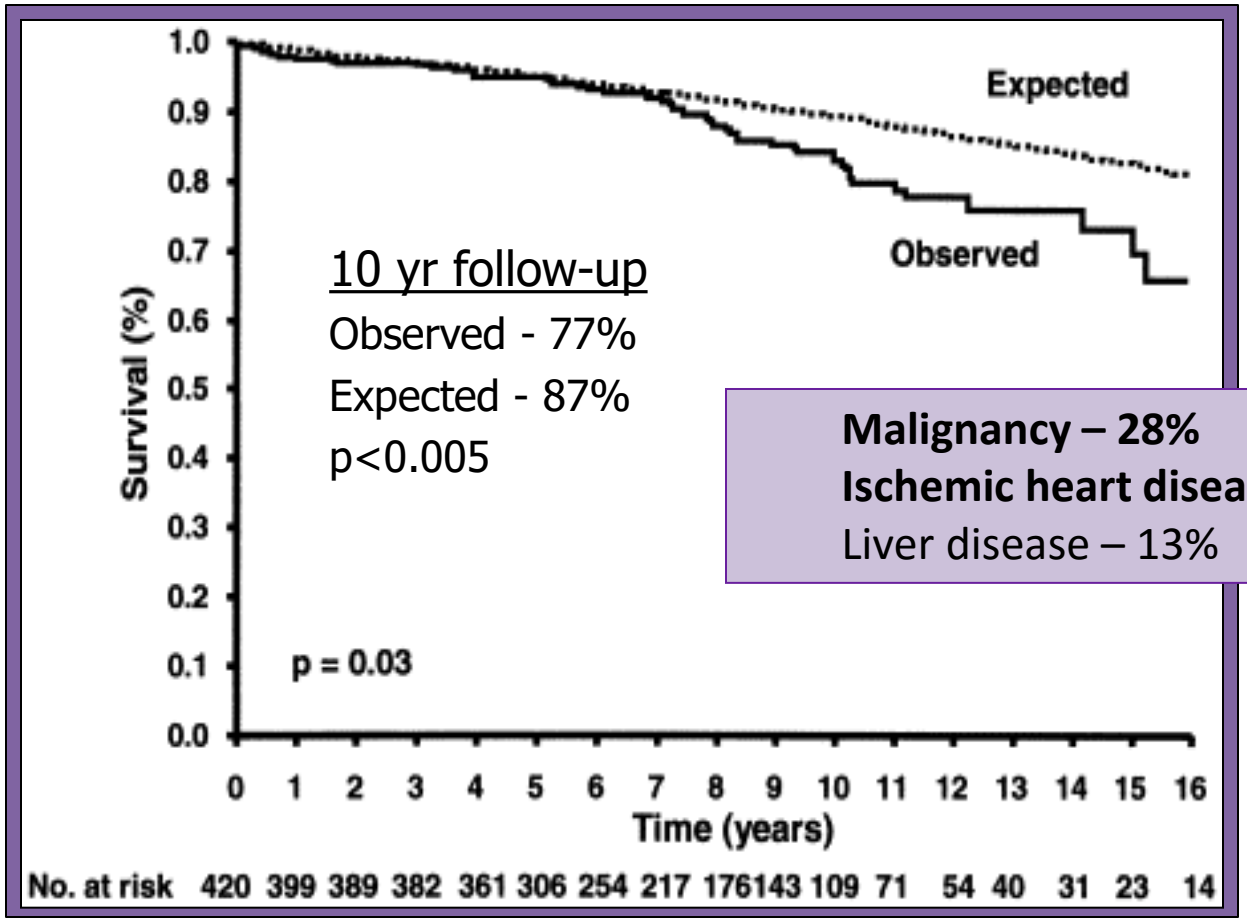
Disclosures

- **Consulting:** Abbvie, Intercept, Fibrogen, NGM Bio, NuSirt, Exhalenz
- **Editorial board:** Hepatology, Seminars in Liver Disease

No conflicts pertaining to content

Mortality in NAFLD

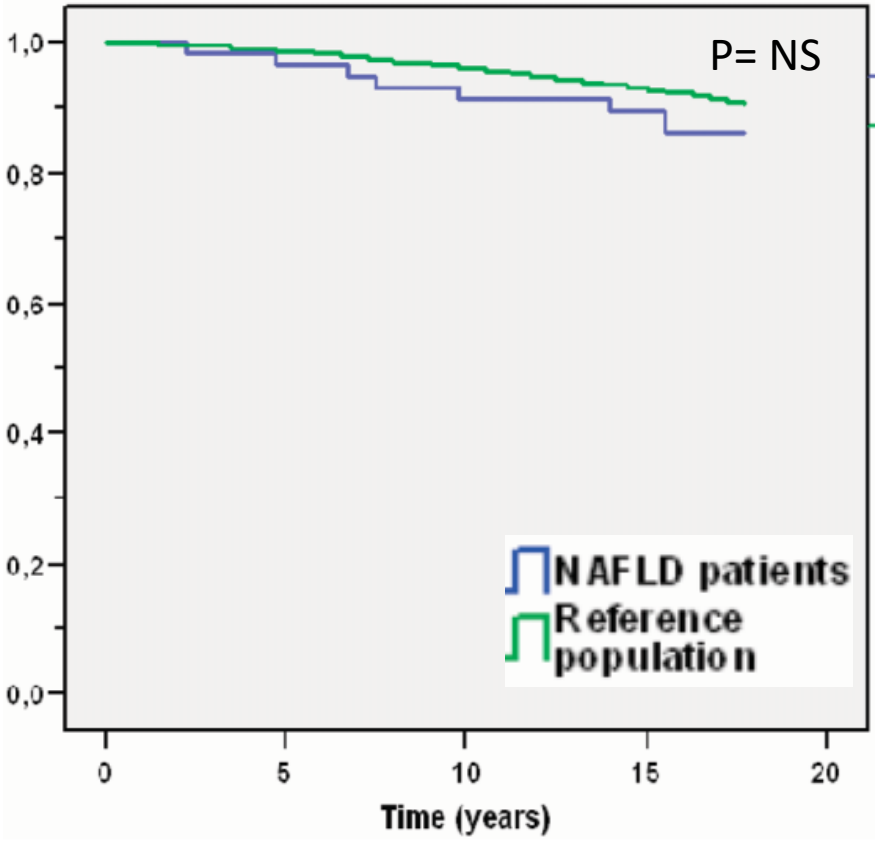


Malignancy – 28%
Ischemic heart disease – 25%
Liver disease – 13%

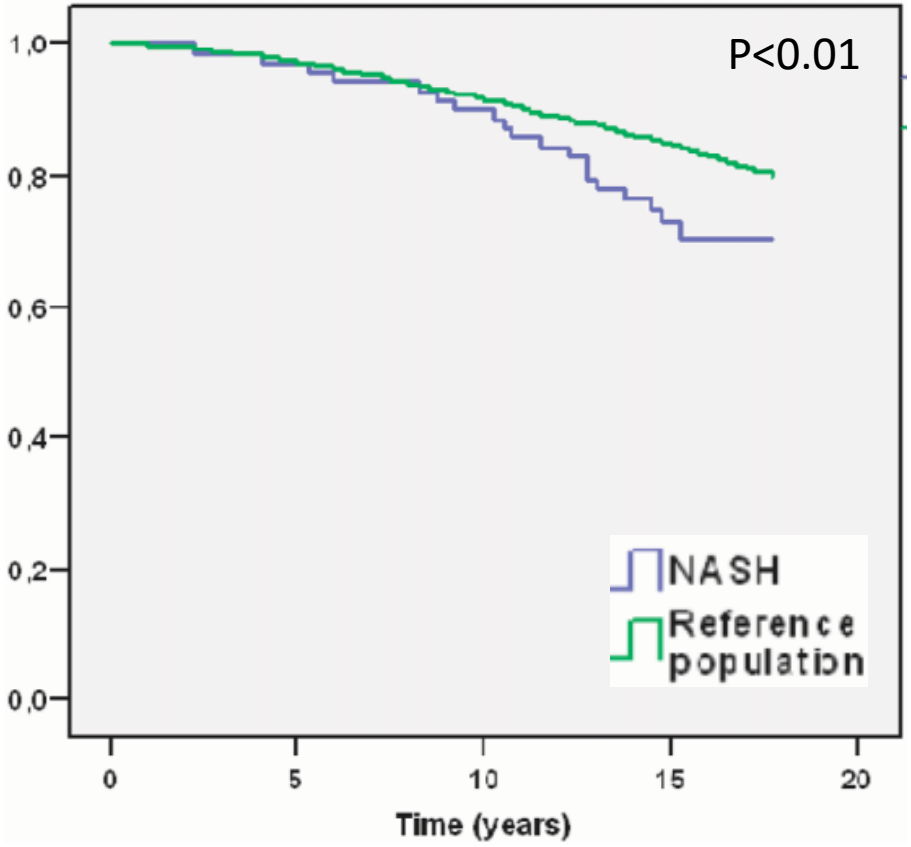
Adams et al., *Gastroenterology* 2005

Mortality due to NASH and isolated hepatic steatosis

Isolated hepatic steatosis



NASH



Ekstedt et al., *Hepatology* 2006

Histologically defined NAFLD and cardiovascular mortality

Author [ref]	N	Follow-up (yrs)	Proportion of deaths due to CVD (%)	Findings
Angulo [5]	619	12.6 (median)	**38.3**	CVD most common COD Extent of fibrosis independently assoc c death
Söderberg [1]	118	24 (median)	**30**	↑Death in those w NASH, CVD most common COD
Ekstedt [2]	129	13.7±1.3 (mean)	**16**	↑CVD death NASH not SS CVD most common COD
Dam-Larsen[4]	170	20.4 (median)	**38**	No difference between SS and control
Rafiq [5]	173	18.5 (median)	**12.7**	CVD death NAFLD=NASH

[1] Söderberg et al., *Hepatology* 2010;[2] Ekstedt et al., *Hepatology* 2006; [3] Adams Dam-Larsen et al., *Scand J of Gastroenterol* 2009; [5] Rafiq et al. *Clin Gastro Hep* 2009; 5 Angulo et al. Gastroenterology 2015

NHANES III suggested that NAFLD did not increase mortality

- Population-based prospective cohort study
- 11,371 patients: 1988-94, follow up mortality to 2006
- Groups:
  - **'Normal'=**No fat by US
  - **'NAFLD'** = Fat on US + normal ALT
  - **'NASH'** = Fat on US + elevated ALT
  - Average age mid-40s
- **No increase in mortality after mean follow-up of 14.6 yrs**

Lazo et al., *BMJ* 2011

NHANESIII: Association between Fibrosis and Overall and Cause-Specific Mortality among Subjects with NAFLD

		Age, Sex-adjusted	Multivariable-adjusted
	n	Hazard ratio (95% CI)	Hazard ratio (95% CI)
Mortality from all cause	778		
Minimal	251	1	1
Intermediate	404	1.50 (1.20-1.88)	1.40 (1.09-1.81)
Advanced	123	2.26 (1.59-3.21)	1.80 (1.23-2.64)
Cardiovascular disease	296		
Minimal	81	1	1
Intermediate	167	2.43 (1.69-3.50)	2.49 (1.71-3.64)
Advanced	48	3.34 (2.00-5.60)	3.22 (1.92-5.42)

Multivariable models adjusted for age, sex, race-ethnicity, education, income, diabetes, hypertension, smoking status, waist circumference, alcohol consumption, caffeine consumption, total cholesterol, high-density lipoprotein-cholesterol, transferrin saturation, and C-reactive protein.

Kim et al. *Hepatology* 2013

Limitations of population based studies

- Well designed to measure CV outcomes
- Imaging or serology alone are not reliable for defining hepatic disease
  - **Allocation**: substantial overlap in comparison groups (*i.e.* limits of detectability, often advanced NASH has less steatosis, lower enzymes)
  - ? negate potential effect of NAFLD/NASH on CV mortality
- Highlights importance of accurate distinction between NAFLD and NASH to assess outcomes

Predicting CVD in patients with NAFLD

- **Framingham Risk Score:** (Age, gender, TC, HDL, smoking and SBP) underestimates risk in the setting of the Metabolic Syndrome[2]
- **Pooled Cohort Equation:** (**FRS** + race, DBP, Rx for HTN, DM)[3]
- **Global risk prediction studies in NAFLD are flawed: derived from traditional CV risk factors**
- ***Factors that are not accounted for in traditional models of cardiovascular risk:***
  - Insulin resistance
  - Triglycerides
  - Obesity

[1] Treeprasertsuk et al., *Liver International* 2012; [2] Dekkar et al., *Circulation* 2005; [3] Stone et al. 2013

Estimated 10 year CVD risk according to NAFLD severity

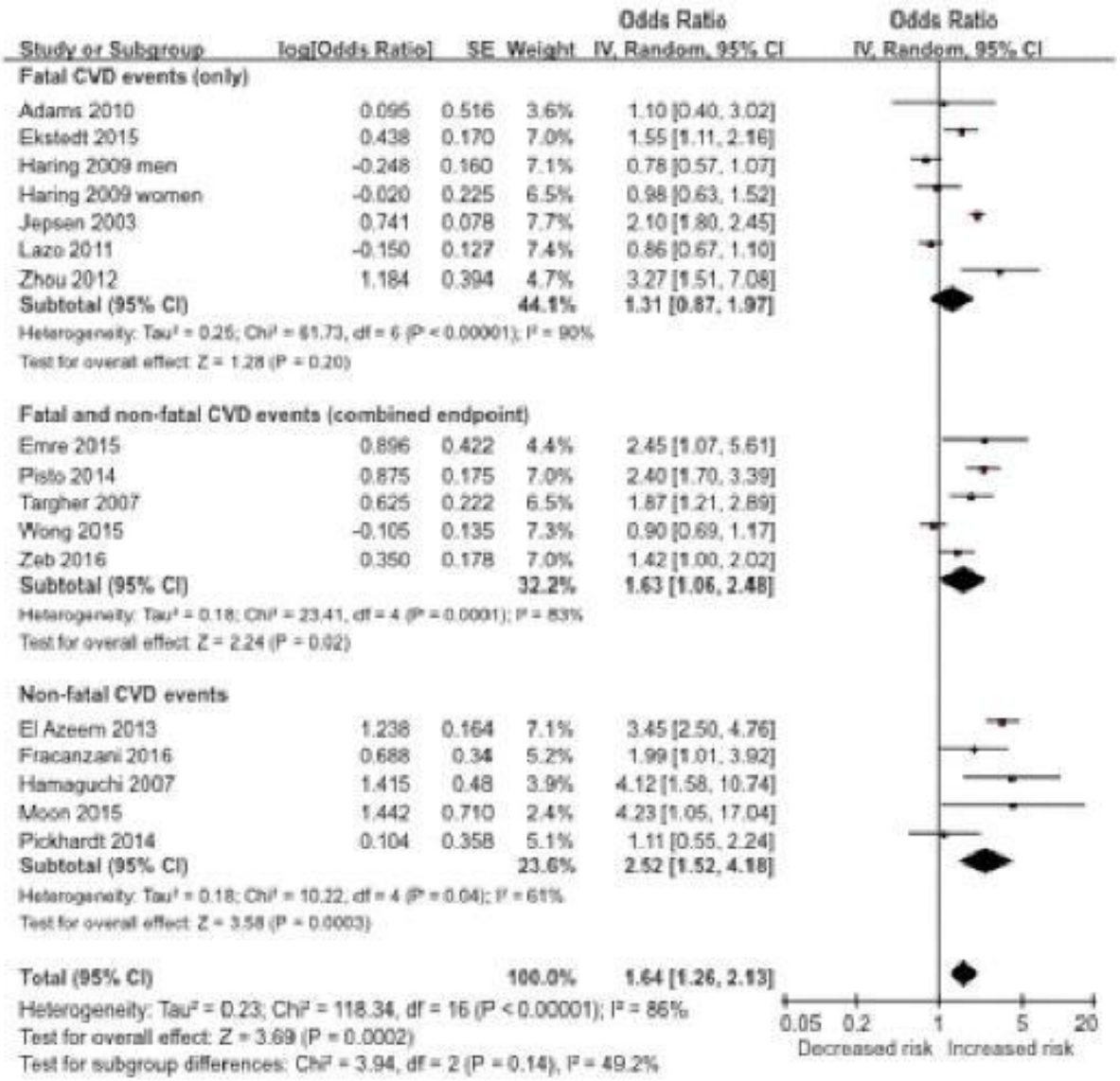
	NAFLD severity				
	None	**Mild**	**Mod**	**Severe**	**P for trend**
Pooled Cohort Equation	2.59	3.93	4.68	5.23	<0.01
Adjusted OR		**1.52 (1.24-1.86**	**2.56 (1.83-3.59)**	**3.35 (1.52-7.29)**	
Framingham Risk Score	4.55	6.39	7.33	7.13	<0.01
Adjusted OR		**1.65 (1.45-1.86)**	**1.62 (1.3-2.01)**	**1.72 (0.93-3.17)**	**<0.001**

OR for >7.5% CVD risk by PCE had stronger correlation with increasing severity of steatosis after adjustment for traditional CV risk factors

Lee et al. Endocrinology and Metabolism 2016

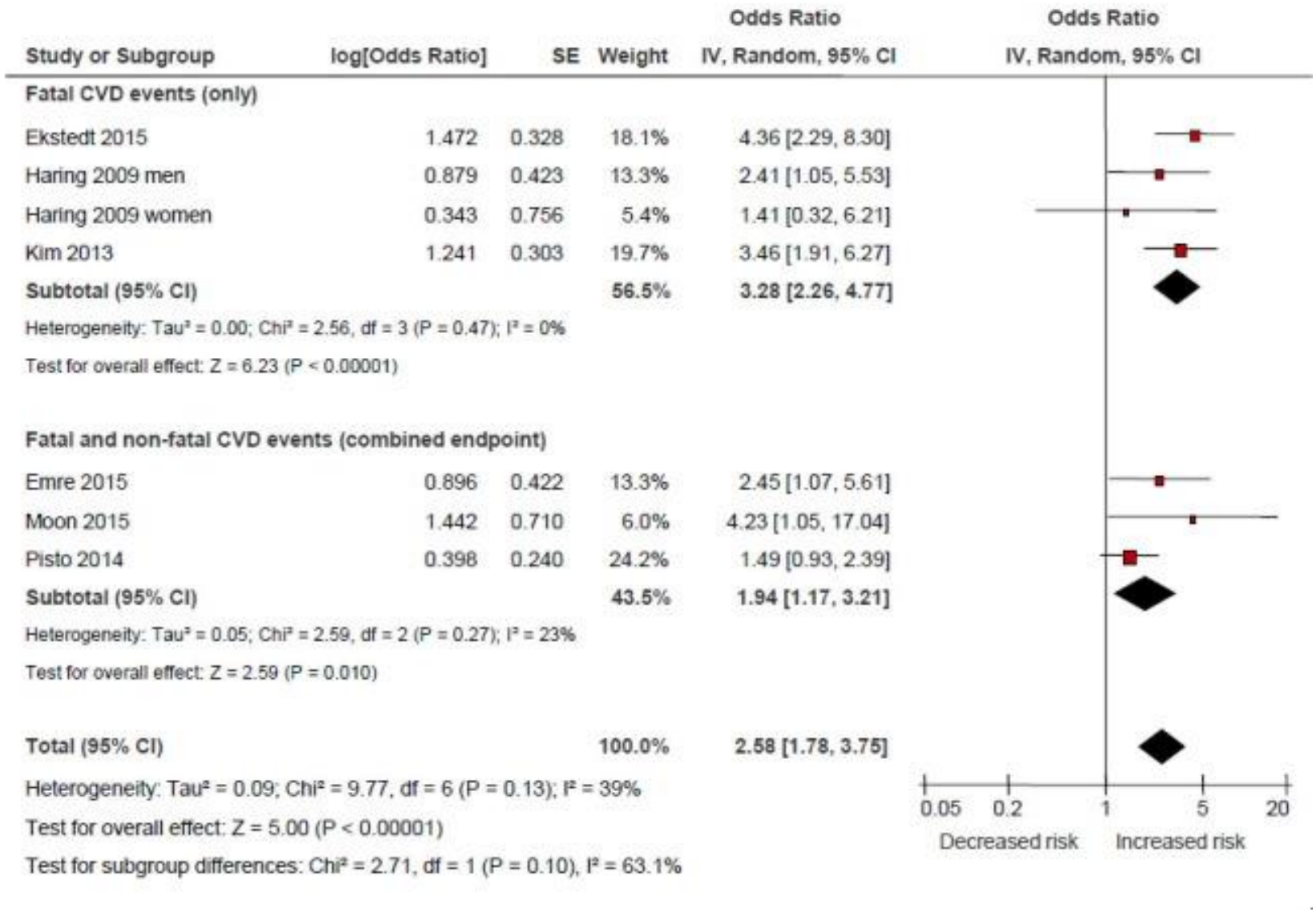
Risk of incident CVD events (fatal, non-fatal or both) associated with NAFLD

- 16 observational studies
- 34,043 adults (36.3% NAFLD)
- ≈2,600 CVD outcomes (>70% CVD deaths)
- Median of 6.9 years



Targher et al. *Journal of Hepatology*, 2016

Risk of incident CVD events (fatal, non-fatal or both) associated with NAFLD severity

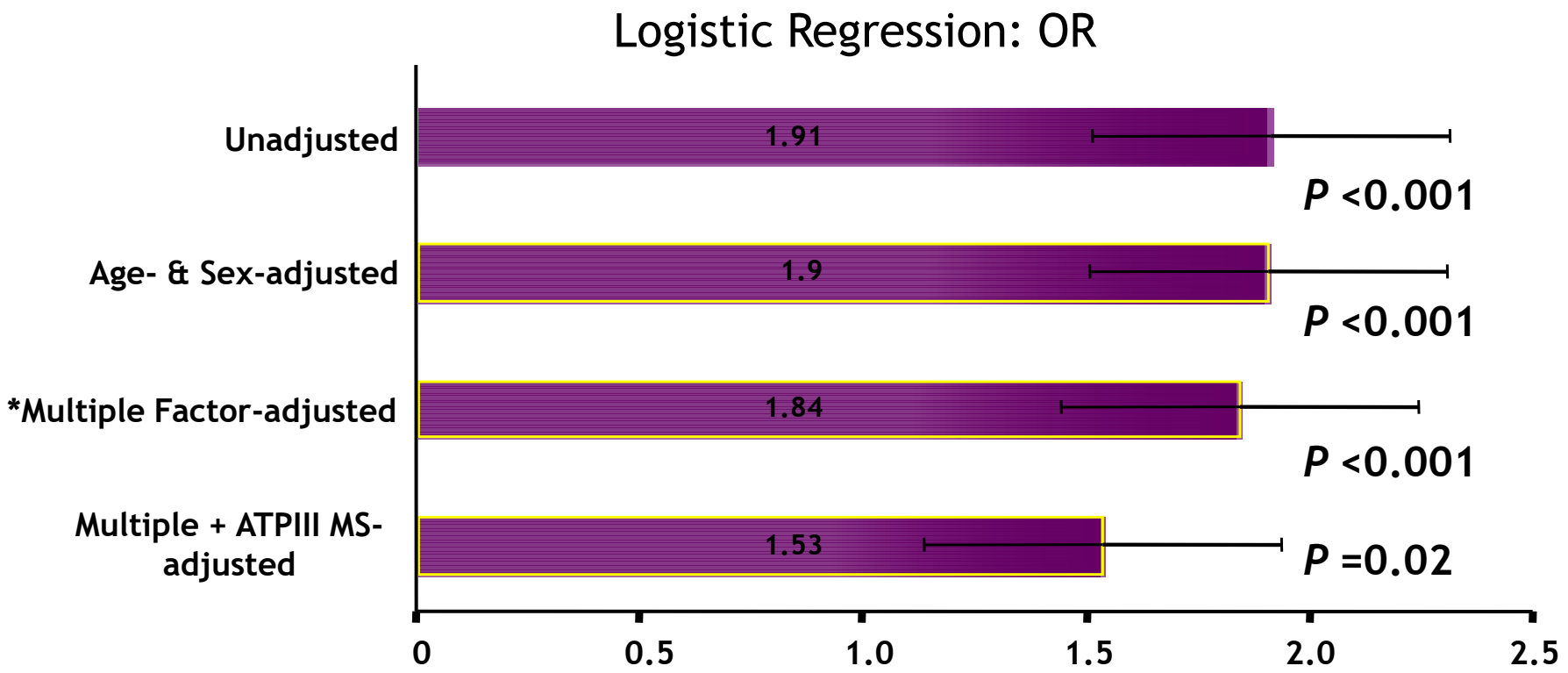


Targher et al. *Journal of Hepatology*, 2016

NAFLD and incident CVD in Type 2 Diabetes

Nested case-control study in 2,103 T2DM, free of CVD at baseline[1].

- 248 cases had a CV event at follow-up (5 yrs), and were compared with 496 who remained free of diagnosed CVD.



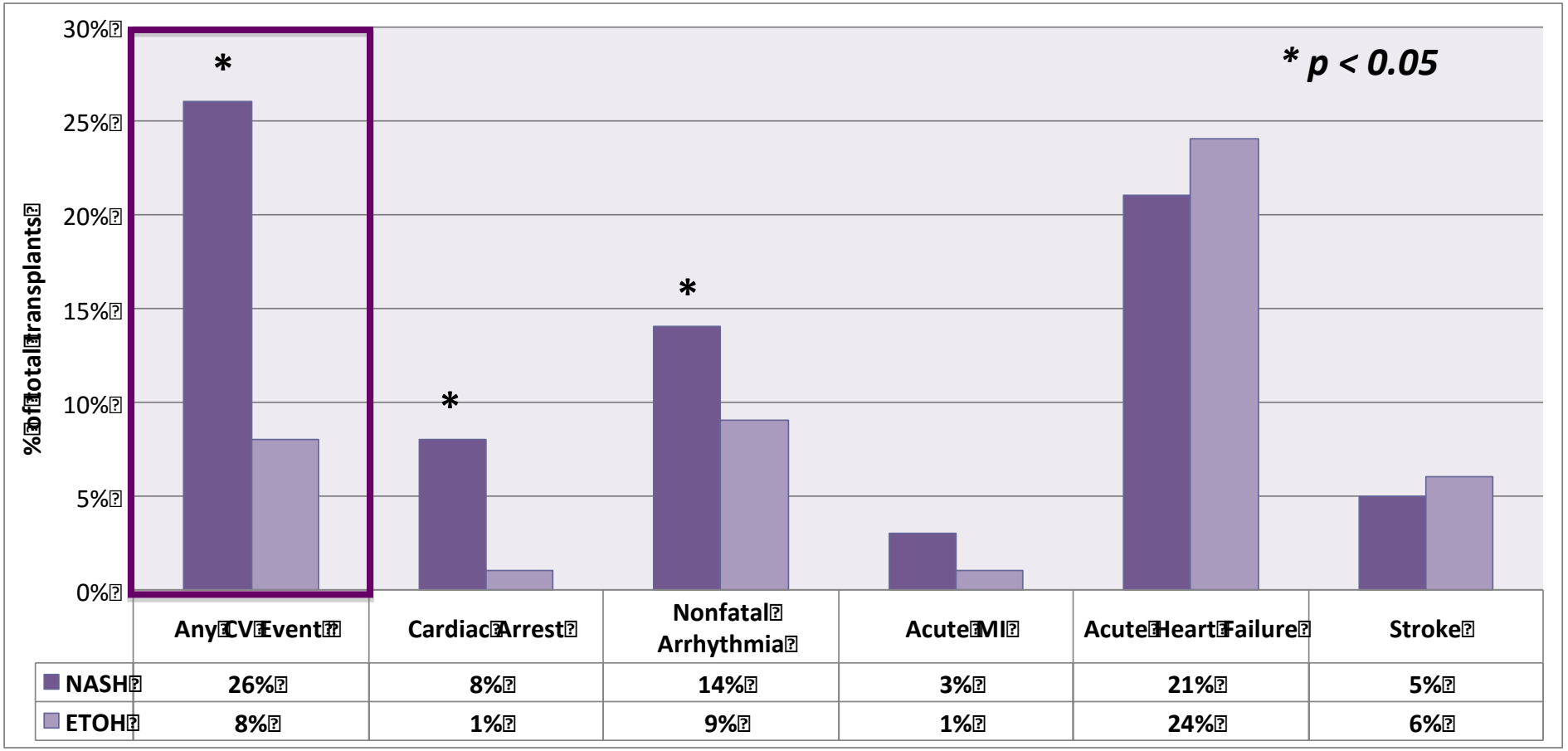
* Age, Sex, Smoking, Duration of diabetes, HbA1c, LDL-cholesterol, drug use (OHA, BP-lowering, statins/fibrates, Aspirin)

Courtesy of AJS

Targher et al., *Diabetes* 2005; Targher et al. Diabetes Care 2007

Cardiovascular Events within 1 year of Liver Transplantation

Revised Cardiac Risk index: Expected event rate: 6.6%

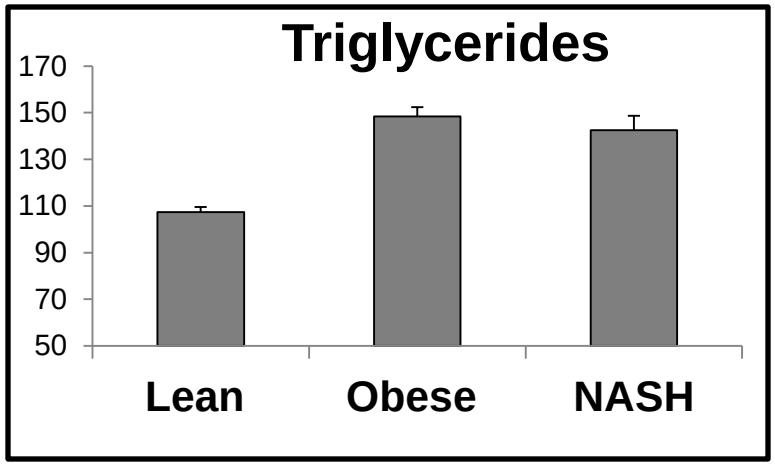


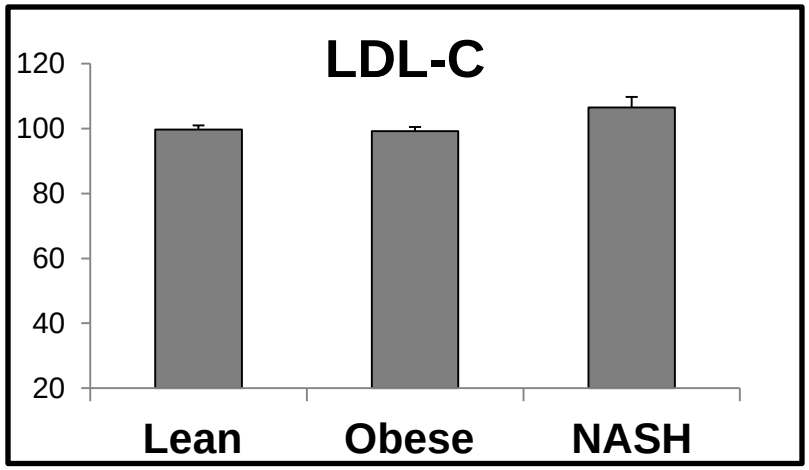
	Any CV Event	Cardiac Arrest	Nonfatal Arrhythmia	Acute MI	Acute Heart Failure	Stroke
NASH	26%	8%	14%	3%	21%	5%
ETOH	8%	1%	9%	1%	24%	6%

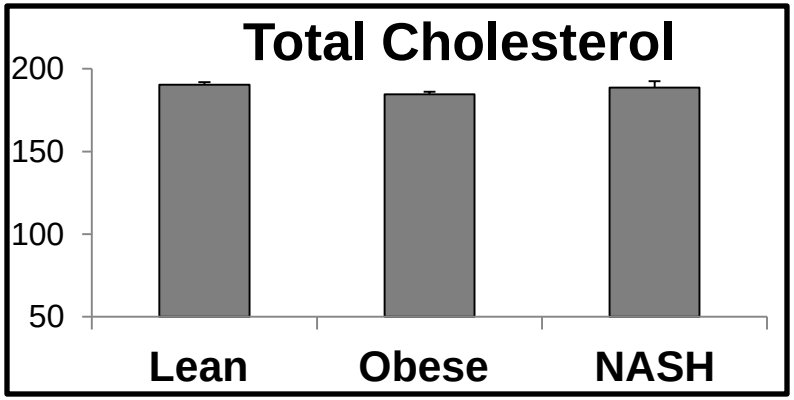
Odds ratio for any CV event: 2.69 (95% CI: 1.32-6.34)

VanWagner, Bhave, Te, Feinglass, Alvarez, Rinella. *Hepatology*. 2012 Nov;56(5):1741-50.

Traditional lipid markers of CV risk are similar between NASH and obese controls







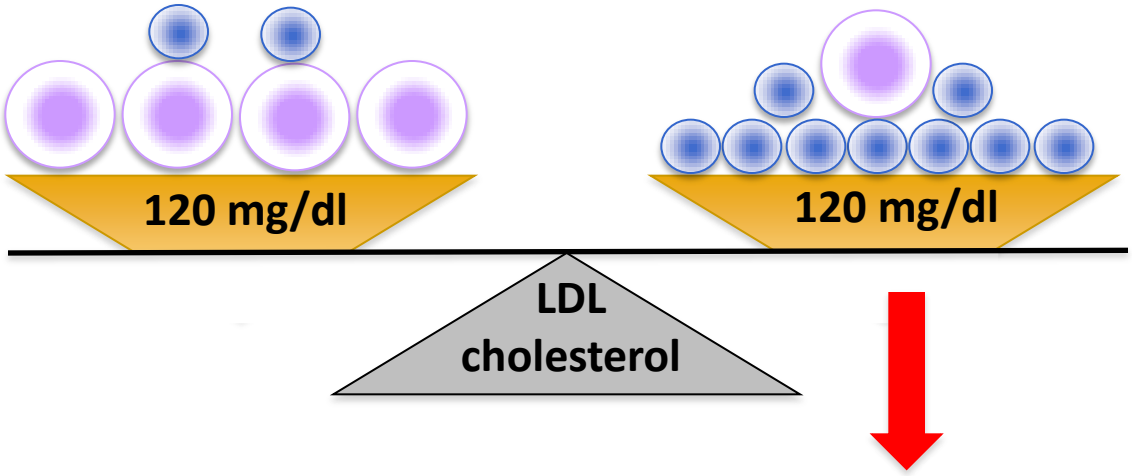
Beyond calculated LDL

Larger particles

Fewer particles

Smaller particles

More particles



Slower plasma clearance
Greater artery uptake & retention
Faster oxidation

Courtesy of AJS

Lipoprotein particle size and NAFLD severity: MESA cohort

Particle size (nm)	No NAFLD N=2793	Mild NAFLD N=432	Moderate NAFLD N=291	Severe NAFLD N=64	Adjusted p-value
VLDL	49.8±8.4	55.3±9.2	56.1±10	59.2±10	<0.001
LDL	20.9±0.77	20.5±0.74	20.4±0.69	20.4±0.79	NS
HDL	9.19±0.42	8.89±0.36	8.85±0.29	8.97±0.39	<0.001
Particle ratios					
LDL small/large	4.3±15	9.8±47	8.5±15	11.2±17	<0.001
HDL small/large	5.6±9.9	9.2±17	8.4±9.1	11.0±14	<0.001

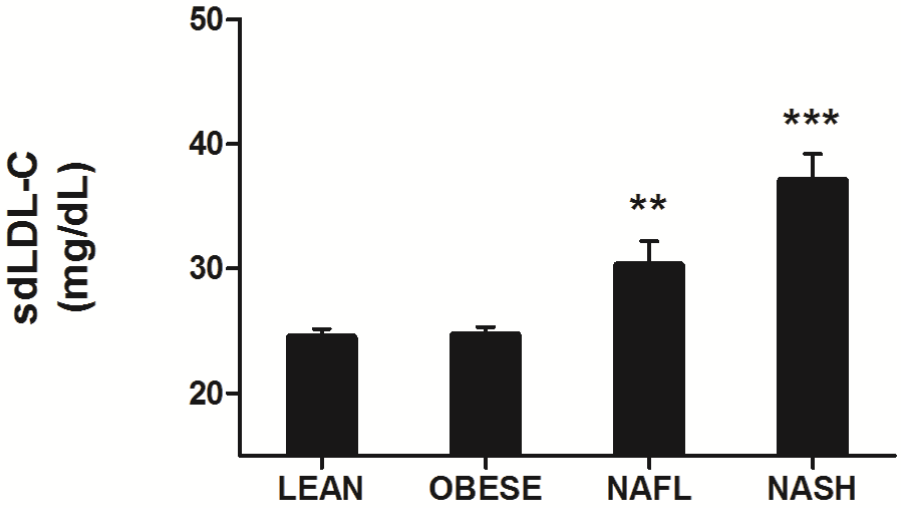
*P values adjusted for age, gender and race/ethnicity. Derived from multivariable robust linear regression model

NAFLD determined by CT

DeFilippis et al., *Atherosclerosis* 2013

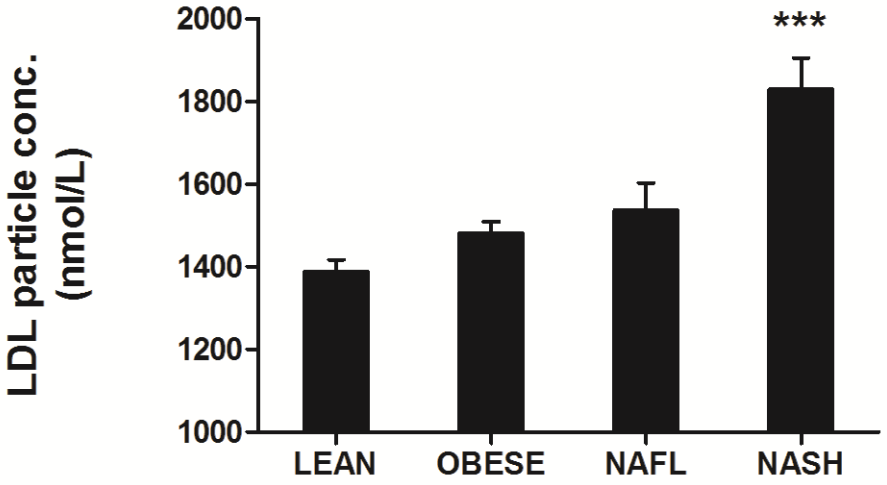
Atherogenic dyslipidemia in lean, obese and NAFLD

Figure 1C



Optimal sdLDL <26%

Figure 1B



Siddiqui et al., *CGH* 2014

Dyslipidemia driven by steatosis and IR not NASH

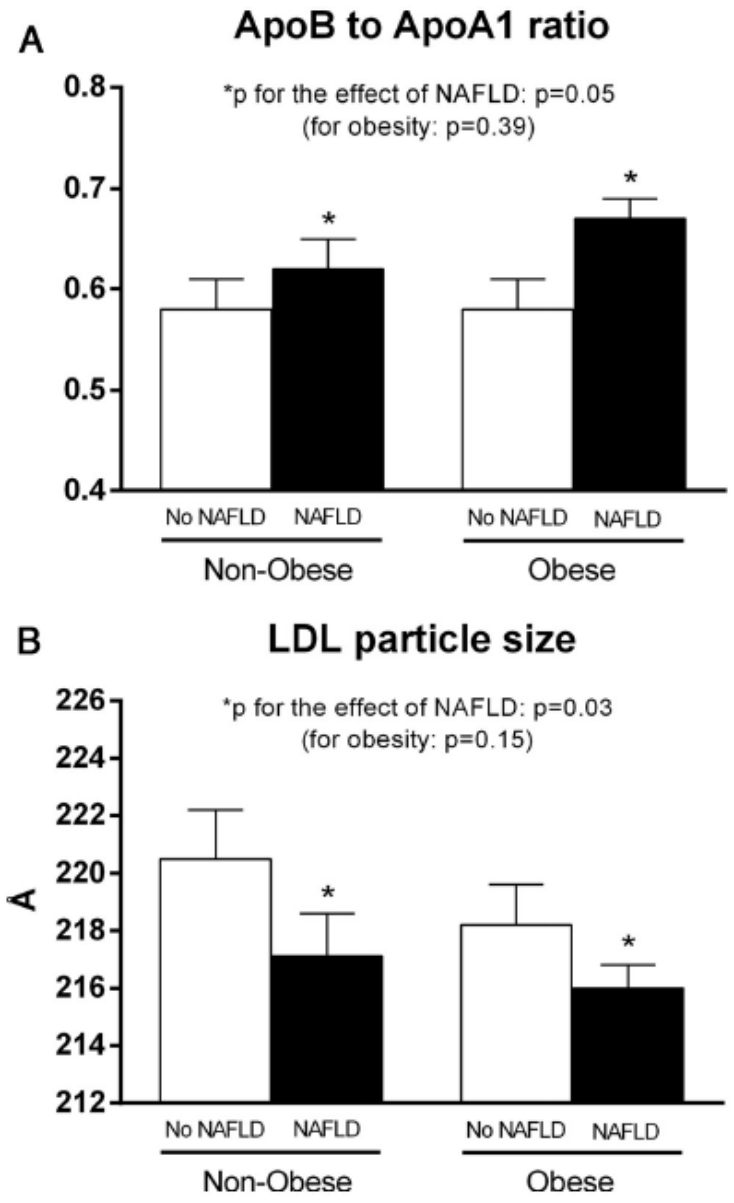


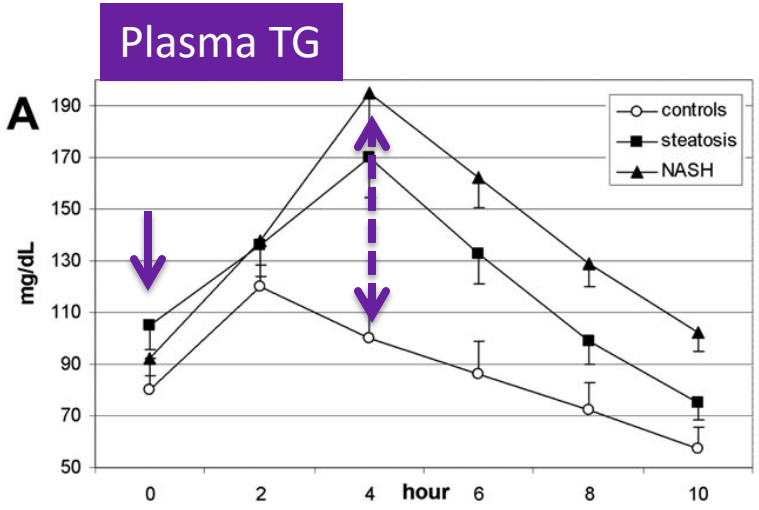
Table 2. Clinical Characteristics of Patients According to the Presence of NASH

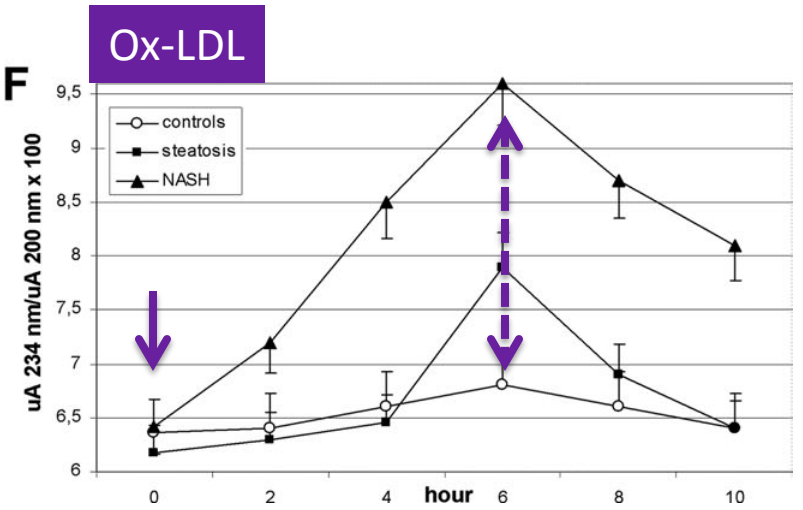
	No NASH (n = 33)	Definite NASH (n = 91)	*P* Value
Age, y	61 ± 1	58 ± 1	.15
Gender (male), %	85%	89%	.54
BMI, kg/m^2	32.8 ± 0.8	35.0 ± 0.4	.01
TBF, %	38 ± 1	37 ± 1	.63
Prevalence of T2DM, %	100%	93%	.34
FPG, mg/dL	142 ± 8	146 ± 4	.63
FPI, μIU/mL	12 ± 1	19 ± 2	.01
Hemoglobin A1c, %	6.9 ± 0.2	7.3 ± 0.1	.09
FFA, mmol/L	0.39 ± 0.03	0.41 ± 0.02	.56
Cholesterol, mg/dL	164 ± 8	172 ± 5	.42
Triglycerides, mg/dL	131 (100–162)	155 (111–234)	.05
LDL-C, mg/dL	93 ± 7	93 ± 4	.95
HDL-C, mg/dL	42 ± 2	39 ± 1	.24
On statins, %	72%	79%	.44
Liver fat, %	13 ± 2	12 ± 1	.58
AST, IU/L	28 ± 2	41 ± 3	.006
ALT, IU/L	34 ± 3	54 ± 4	.003

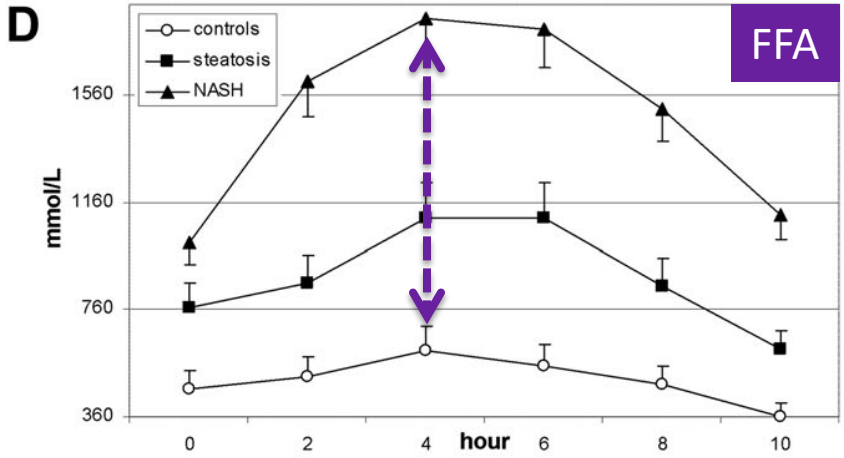
Abbreviations: ALT, aminotransferase; AST, aspartate aminotransferase; FPG, fasting plasma glucose; TBF, total body fat.

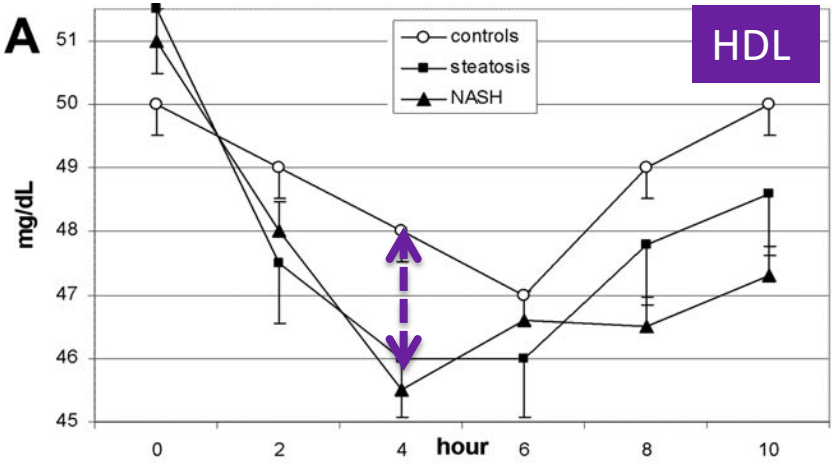
Bril et al., *JCEM* 2016

Pro-atherogenic post prandial lipid metabolism









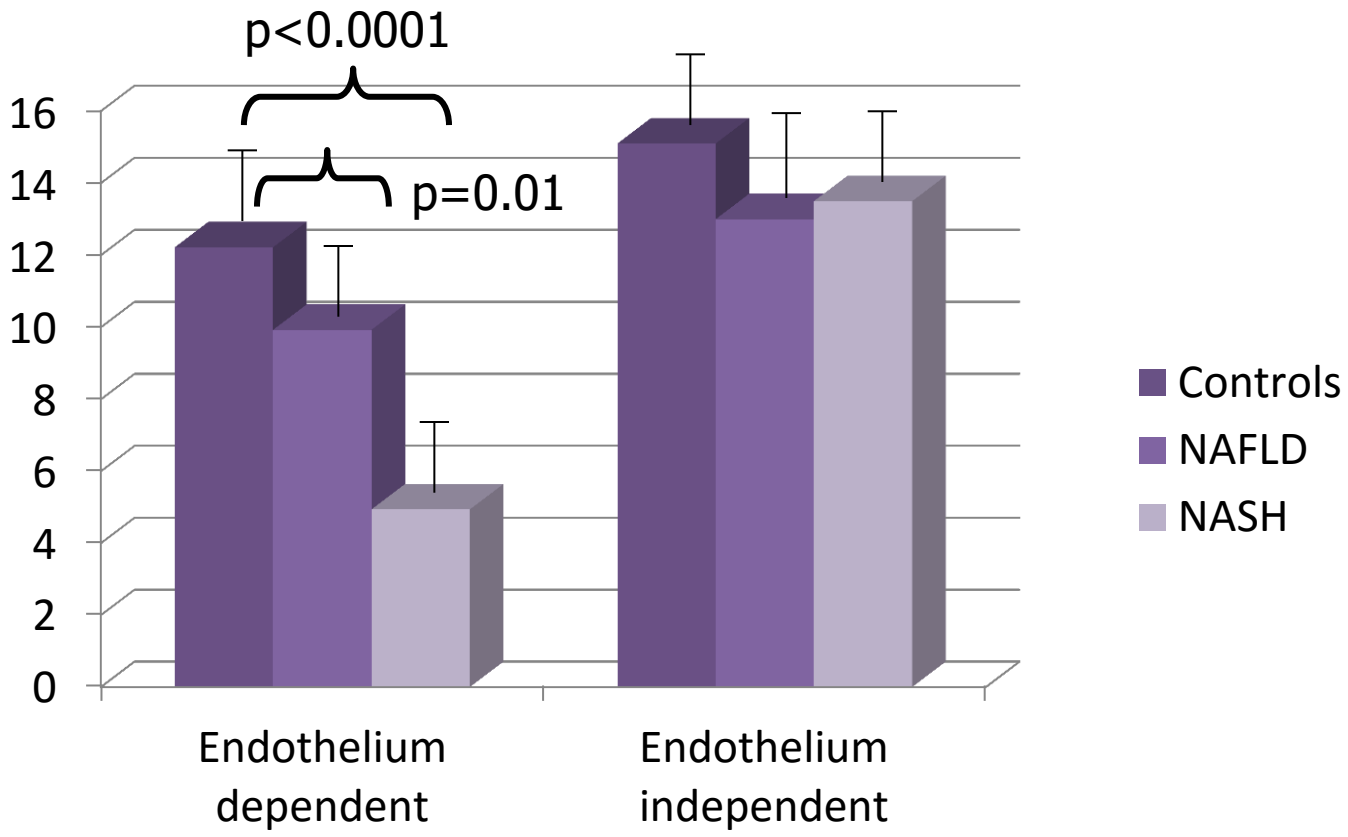
Musso et al., *Hepatology* 2012

Development of atherosclerosis

- **Sub-clinical**
  - Endothelial dysfunction
  - Carotid intima media thickening
  - Coronary artery calcium scores
  - Impaired coronary flow reserve

Dysfunctional Endothelium in fatty liver

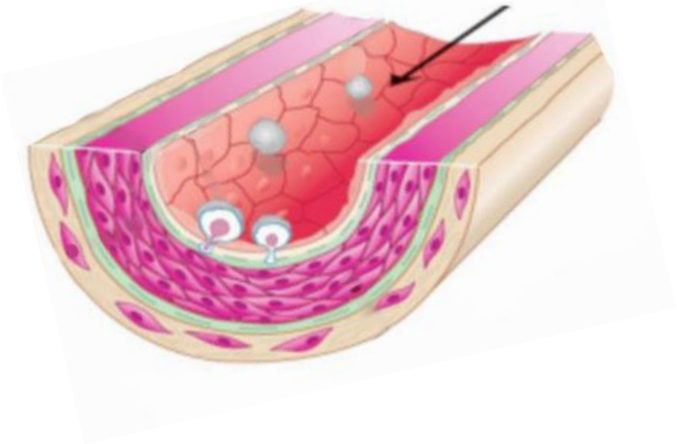
- 52 NAFLD cases with age/sex matched controls
- FMV (controlling for BMI, IR and cardiac risk assessment by FRS calculated [1]



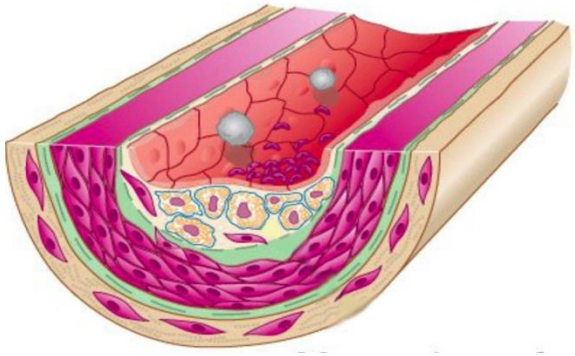
[1] **Villanova et al., *Hepatology* 2005; Salvi et al. J Hypertension 2010; Pacifico et al. Hepatology 2010**

Development of Atherosclerosis

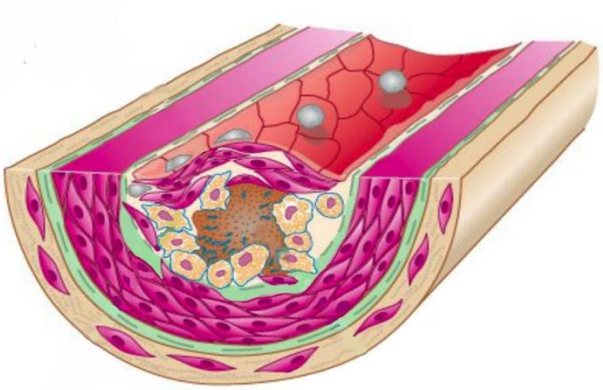
Normal



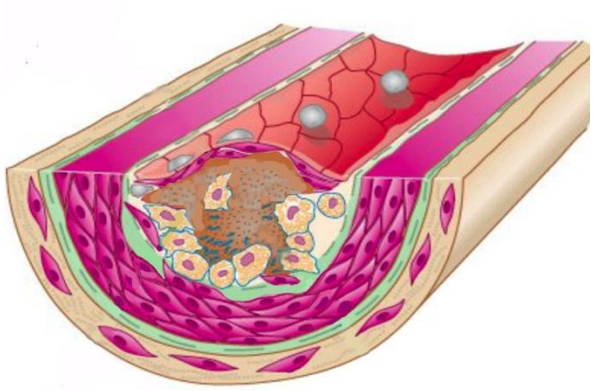
Pathological intimal thickening



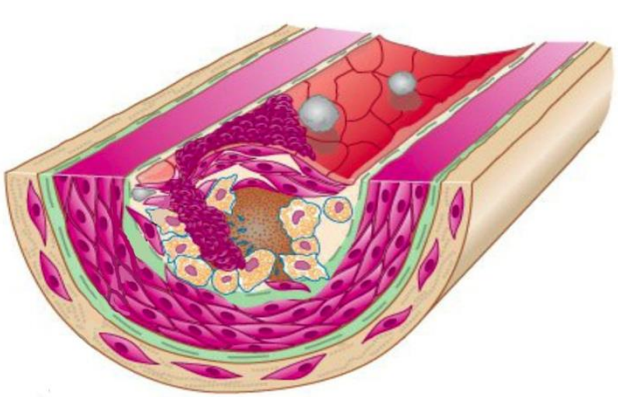
Fibrous cap atheroma



Thin cap atheroma

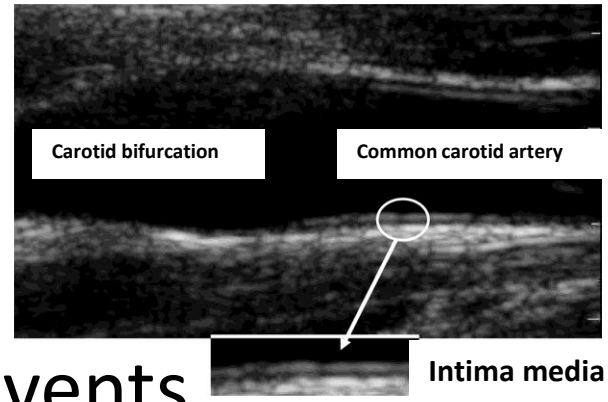


Plaque rupture



NAFLD and carotid intima-media thickening (CIMT)

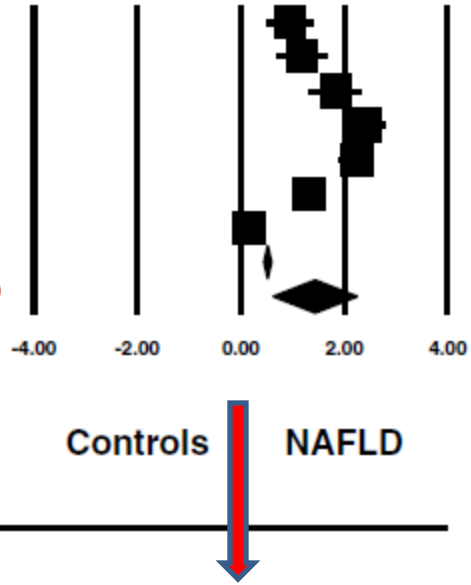
- Well validated tool to detect atherosclerosis in asymptomatic patients
- Independently predicts CVD events
- Improves risk prediction for CVD when added to Framingham risk factors
- Several studies have shown an association with NAFLD though this is less convincing after adjusting for MetS and other confounders



Sonoda et al., *Int J Cardiol* 2004; Fernandes et al., *J Amer Coll Cardiol* 2006; Polak et al. *JAHA* 2013

CIMT strongly associated with NAFLD

Model	Study name	Outcome	Statistics for each study				Sample size		
			Std diff in means	Lower limit	Upper limit	p-Value	NAFLD	Controls	Total
	Brea A et al, 2005	IMT	0.9486	0.4863	1.4108	0.000057692	40	40	80
	Aygun C et al, 2008	IMT	1.1902	0.7147	1.6656	0.000000930	40	40	80
	Targher G et al, 2004	IMT	1.8323	1.3251	2.3395	0.000000000	45	40	85
	Targher G et al, 2006b	IMT	2.4083	2.0443	2.7724	0.000000000	100	100	200
	Targher G et al, 2006	IMT	2.2421	1.9125	2.5716	0.000000000	85	160	245
	Fracanzani et al, 2008	IMT	1.3258	1.0911	1.5605	0.000000000	125	250	375
	Volzke H et al, 2005	IMT	0.1577	0.0767	0.2387	0.000135208	992	1440	2432
Fixed			0.5179	0.4472	0.5885	0.000000000	1427	2070	3497
Random			1.4391	0.6320	2.2462	0.000474500	1427	2070	3497

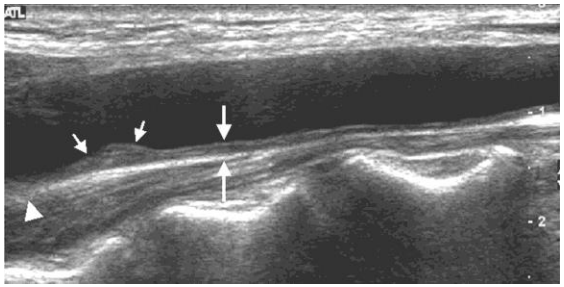


Intima-media thickness is strongly associated with NAFLD: Patients with NAFLD (n: 1427) have an increase of 13% of IMT in comparison with individuals without fatty liver (n:2070)

Courtesy of S. Sookoian

Higher prevalence of carotid plaques in NAFLD

Model	Study name	Outcome	MH odds ratio	Lower limit	Upper limit	p-Value	NAFLD	Controls	Total
	Brea A et al, 2005	Plaques	3.2222	1.2681	8.1879	0.013929452	40	40	80
	Aygun C et al, 2008	Plaques	4.6782	1.1935	18.3370	0.026844845	40	40	80
	Targher G et al, 2006	Plaques	4.3322	2.4621	7.6226	0.000000367	85	160	245
	Fracanzani et al, 2008	Plaques	4.1145	2.0897	8.1011	0.000042741	125	250	375
	Volzke H et al, 2005	Plaques	1.6617	1.3829	1.9967	0.000000060	992	1440	2432
Fixed			1.9735	1.6742	2.3263	0.000000000	1282	1930	3212
Random			3.1325	1.7575	5.5831	0.000107776	1282	1930	3212

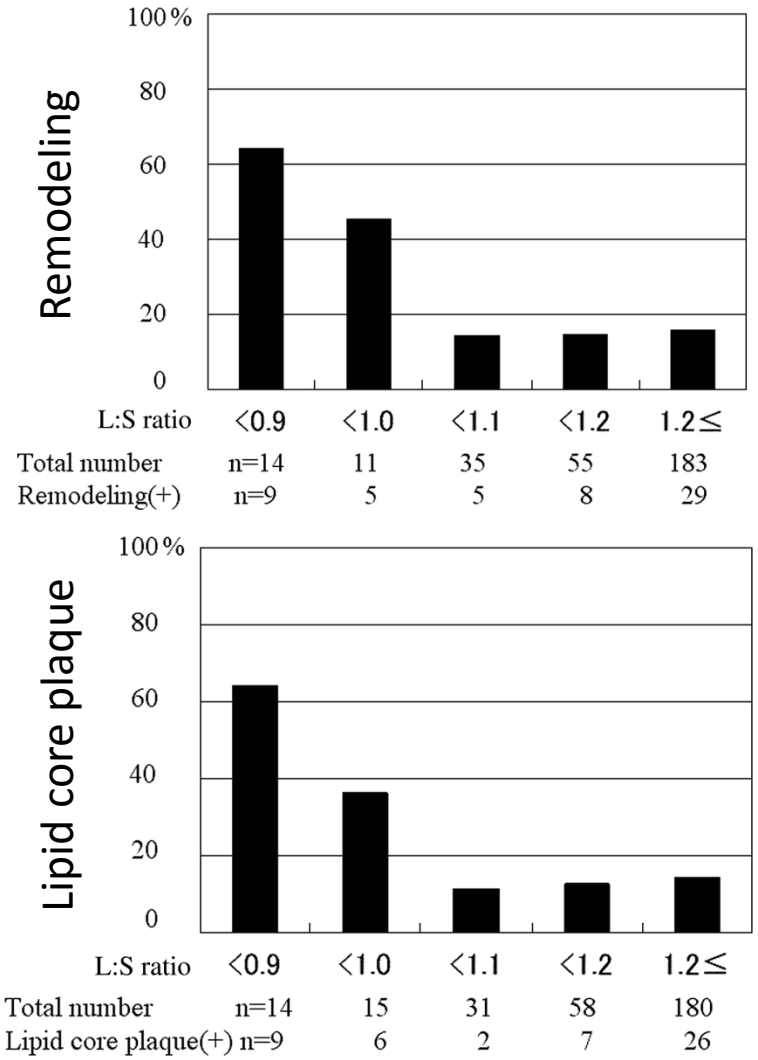


The comparison between cases (n: 1282) and controls (n:1930) showed that carotid plaques were more frequently observed in NAFLD patients (OR 3.13 CI 95% 1.75-5.58, p<0.0002 random model)

Courtesy of S. Sookoian

Sookoian S and Pirola CJ, *Journal of Hepatology* 2008

NAFLD is associated with increase in vulnerable plaque and overt CAD

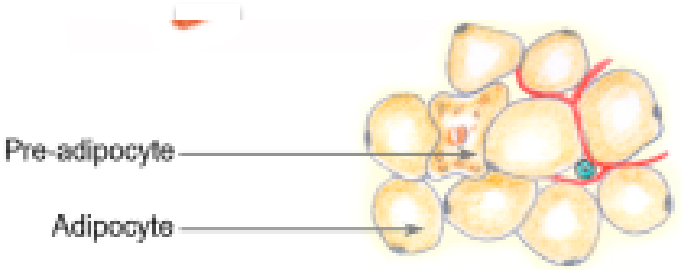


- Independent of:
  - Age
  - Gender
  - Smoking
  - BMI
  - HTN
  - Impaired fasting glucose/DM
  - Dyslipidemia

Akabame et al., *Circ J* 2008

Adipose tissue expansion – is it the nidus of the problem?

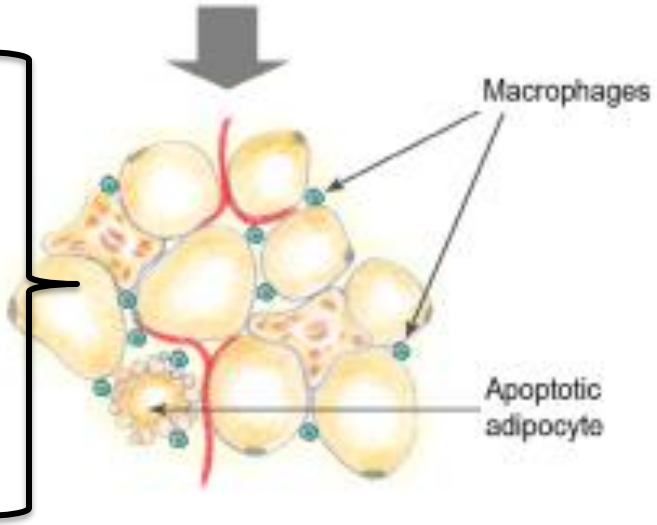
Liver is also a source of cytokines and pro-inflammatory factors *



↑↑Fatty acid synthesis
↑De novo lipogenesis
↑ β-oxidation

Oxidative injury
Apoptosis
Inflammation
Fibrogenesis

Pro-inflammatory cytokines
•TNF-α
•IL-6
•MCP-1
Adipocytokines
•Adiponectin (🡻)
•Leptin
•Resistin
Macrophage derived factors
•IL-1β



Adapted from ***Pillai & Rinella, Clin of North America 2010***

Multiple sources

Adipose tissue insulin resistance in patients with NAFLD

- 40 patients with NAFLD (20 isolated steatosis, 20 NASH) **and no obesity, DM or dyslipidemia**
- Matched for adiposity and features of the Metabolic syndrome
- **Aim:** to determine if adipose tissue dysfunction mediated liver disease progression and cardiometabolic risk in NAFLD independent of obesity

Musso et al., *Hepatology* 2012

NASH: Higher adipose IR and expression of pro-atherogenic markers independent of adiposity or MetS

	Controls	Isolated steatosis	NASH	P value
Adipo-IR index	17.1+1.9	49.5+403*	82.4+8.2**	0.0003
E-selectin	18.5+2.3	25.3+2.4**	45.9+2.8**	0.004
ICAM-1 mg/mL	194.2+803	239.4+8.2*	279.1+9.3**	0.029
CRP mg/mL	1.2+0.5	1.9+1.1*	2.7+1.2**	0.029
NT mmol/mL	5.1+4.9	16.1+9.2**	27.8+15.3**	0.012

Endothelial dysfunction

Nitrosative stress

*p<0.05, **p<0.01 isolated steatosis vs. controls, NASH vs. isolated steatosis

Musso et al., *Hepatology* 2012

NASH: Independent association with pro-coagulant and inflammatory factors

- **Circulating levels of inflammatory markers**
  - CRP, IL-6, MCP-1, TNF-α
- **Pro-coagulant factors**
  - ↑PAI-1[2,3], fibrinogen, Factor VII, ETP-ratio[5], Factor VIII[5],
  - ↓Protein C [4,5]*,
- **Markers of oxidative stress**

Circulating levels increase from controls to IHS to NASH in a stepwise fashion

*Compared NAFLD to controls

[1] Targher *NEJM* 2011;[2] Targher *Semin Thomb Hemost* 2009; [3]Thuy *J Nutr* 2008; [4]Bell *J Hepatol* 1992; [5]Tripodi J. Hepatology 2014

Differential hepatic expression of atherosclerosis genes in patients with NASH vs. isolated steatosis

Role	Gene	NASH *	P value
Atherosclerosis risk	PAI2	2.1	<0.007
	TGFβ1	3.8	<0.008
CV risk	ACE	2.1	<0.007
Inflammation/cytokine signaling	CSF2	2.5	<0.002
	IL1A	2.5	<0.005
	IL3	2.1	<0.007

* Fold change compared to isolated steatosis

Sookoian et al., *Atherosclerosis* 2011

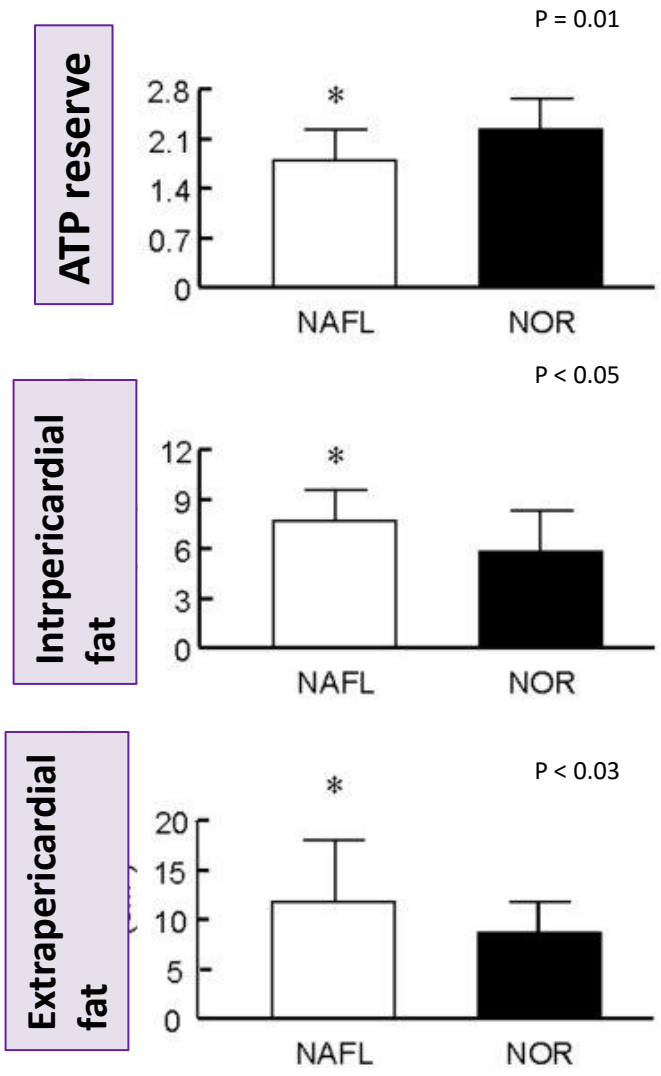
Effects of NAFLD on Cardiac Structure and Function

Increased mediastinal fat and left ventricular energy metabolism in NAFLD

- Young non-diabetic men with hepatic steatosis and matched controls without steatosis
  - Intra- and extra-pericardial mediastinal fat content
  - Left ventricular morphology
  - Left ventricular systolic and diastolic function
  - Resting LV energy metabolism

Perseghin et al., *Hepatology* 2008

Increased mediastinal fat and left ventricular energy metabolism in NAFLD



- Non-obese, no HTN young men with/without NAFLD
- Normal cardiac morphology and function
- At rest NAFLD patients have impaired LV ATP reserve
- Men with fatty livers have more intrapericardial and extrapericardial fat

Perseghin et al., *Hepatology* 2008

Impaired LV function in NAFLD

- Tissue doppler imaging for LV systolic and diastolic function in 35 patients with NAFLD (no DM or HTN)
- 30 healthy controls
- Diagnosis of NAFLD made by ultrasound standard criteria

Fotbolcu et al., *Cardiology Journal* 2010

Echo data in NAFLD vs. Controls

Parameters	NAFLD-patients (n = 35)	Controls (n = 30)	P
IVS [cm]	0.98 ± 0.08	0.79 ± 0.07	< 0.001
PW [cm]	0.93 ± 0.09	0.75 ± 0.07	< 0.001
LVESD [cm]	3.19 ± 0.34	3.18 ± 0.23	0.913
LVEDD [cm]	4.87 ± 0.48	4.76 ± 0.25	0.243
EF (%)	63.40 ± 4.16	62.47 ± 4.31	0.379
LVM [g]	169.83 ± 39.81	114.77 ± 16.43	< 0.001
LVMI [g/m^3]	82.06 ± 16.88	59.17 ± 8.75	< 0.001
LA [cm]	[illegible]	± 0.34	0.113
S′ [cm/s]	[illegible]	± 1.5	0.004

NAFLD — non-alkoholic fatty liver dis[illegible]r wall diastolic thickness; LVESD — left ventricular end-systolic diameter; LV[illegible]M — left ventricular mass; LVMI — left ventricular mass index; LA — left atria[illegible]graphy

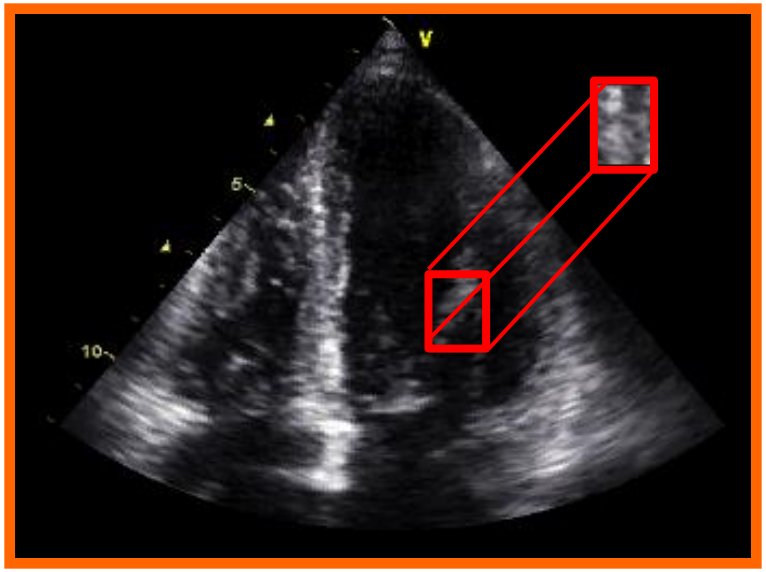
Too small of a study to determine if these effects were independent of other differences between groups i.e. BMI, HTN, IR

Parameters	NA[illegible]	= 30)	P
E [cm/s]	71.1 ± 11.2	74.9 ± 13.5	0.363
A [cm/s]	58.2 ± 9.2	54.3 ± 9.1	0.279
DT [ms]	192.8 ± 33.4	166.7 ± 34.2	< 0.001
IVRT [ms]	107.3 ± 12.1	94.8 ± 12.6	< 0.001
E/A ratio	1.25 ± 0.28	1.42 ± 0.34	0.028
E′ [cm/s]	11.1 ± 2.1	15.3 ± 2.7	< 0.001
E/E′ ratio	6.64 ± 1.39	4.91 ± 0.91	< 0.001

NAFLD — non-alkoholic fatty liver disease; E — early diastolic filling velocity; A — late diastolic filling velocity; DT — deceleration time; IVRT — isovolumic relaxation time; E′ — early diastolic velocity on tissue Doppler echocardiography

Fotbolcu et al., *Cardiology* J 2010

Echocardiographic Speckle (tissue) tracking



- **Myocardial strain :** Analysis of cardiac motion in regions of interest (% change)
- **Global longitudinal strain:**
  - Reflects sub-endocardial function
  - Most susceptible to injury

Adjusted odds ratios for the association of NAFLD with severely impaired global longitudinal strain*

CARDIA
Coronary Artery Risk Development in Young Adults

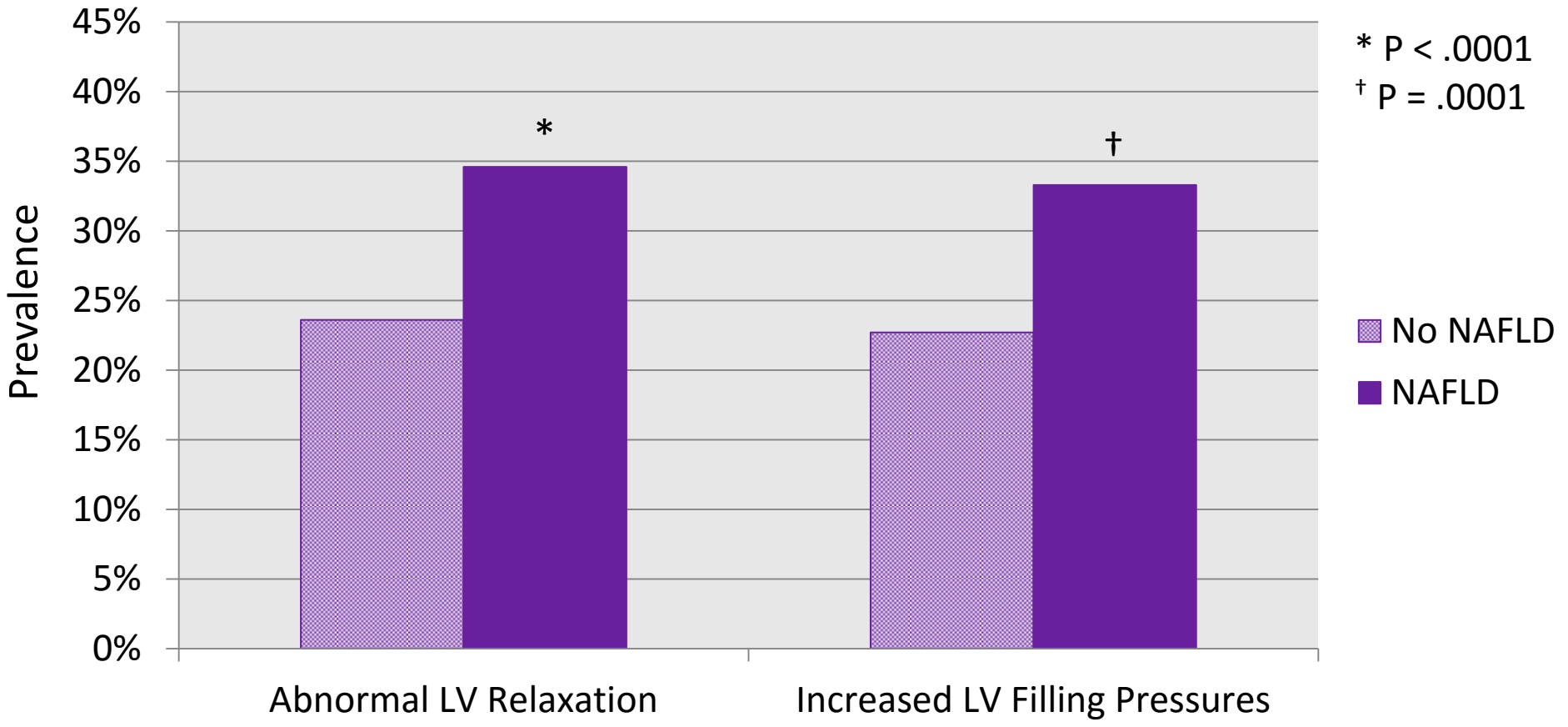
	OR	95% CI	P value
Base model	**3.4**	**2.1-5.5**	**< .0001**
Base + HF risk factors	2.0	1.2-3.2	<.0001
Base + BMI	2.3	1.4-3.7	<.001
Base + VAT	1.8	1.1-3.0	.03

Base Model: age, race, sex, center, alcohol, smoking and physical activity

HF risk factors: systolic BP, anti-HTN & anti-hyperlipidemic medication use, total cholesterol, HDL cholesterol, diabetes status, GFR

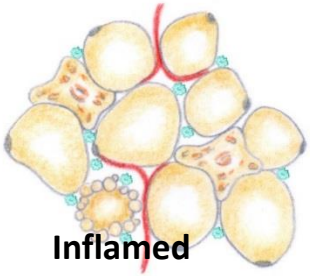
Van Wagner et al. Hepatology 2015

Markers of subclinical diastolic dysfunction in NAFLD participants compared to non-NAFLD



lateral tissue Doppler e' velocity < 10 cm/s.

lateral E/e' ratio ≥ 12 or left atrial volume index ≥ 34 ml/m^2.



Inflamed Visceral Adipose Tissue

Fatty acid synthesis
De novo lipogenesis
β-oxidation

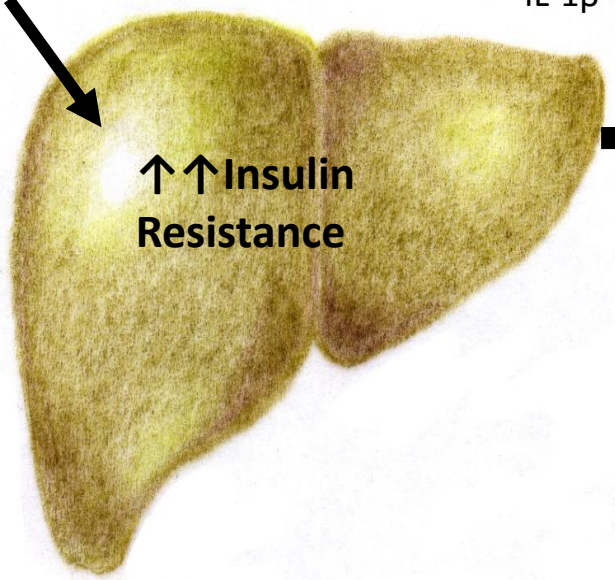
Oxidative injury
Apoptosis
Inflammation
Fibrogenesis

Lipolysis

Myocardial steatosis

Pro-inflammatory cytokines
- •TNF-α
- •IL-6
- •MCP-1
- •Adiponectin (↓)
- •Leptin

Macrophage derived factors
- •IL-1β



↑↑Insulin Resistance

Endothelial Dysfunction
- Impaired NO synthesis

Coagulation Derangements
- PAI-1
- Fibrinogen
- V-WF

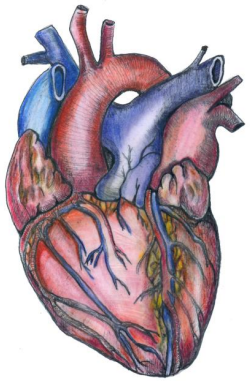
Atherogenic Dyslipidemia
- Altered LDL-c
- sdLDL
- Increased VLDL
- Low HDL -c

Increased gene expression of mediators of atherogenesis
- ACE
- Cytokine Signaling- CF2, IL1A, IL3
- ↑Endothelin 1
- ↑Angiotensinogen
- TGFβ1

Endothelial cells

eNOS

L-arginine → NO



Cardiovascular disease

- Atherosclerosis
- Heart failure
- Arrhythmias

Conclusions

- Association between NAFLD (NASH) and CVD events and mortality is robust
- Emerging association with impaired cardiac function and arrhythmias
- Plausible MoA link NAFLD (NASH) to CVD
- Independent contribution of NAFLD to development and progression of CVD is compelling
- Good practice to incorporate CVD risk reduction strategies in patients with NAFLD as part of a multidisciplinary approach


Thank you