“Is fatty liver a cardio-metabolic risk factor?” A methodological perspective

Giorgio Bedogni
Liver Research Center
Basovizza (TS), Italy

Question

- What are the main methodological problems encountered when trying to establish a causal association between fatty liver (FL) and cardiometabolic disease?

Prelude
What is a “cause”?

- Philosophical debate is ongoing from at least 2 millennia
  e.g. http://en.wikipedia.org/wiki/Cause

- The concept is outside the language of Mathematics
  e.g. Baber RL. The Language of Mathematics. Wiley, 2011.

- Epidemiologists give an operational definition of “cause” on the basis of a practically relevant association with an “effect” of interest
  e.g. Vineis P. Int J Epidemiol 2009;38:675.

A very short summary of the available evidence about FL and cardiometabolic disease

- FL may be an independent risk factor for incident type 2 diabetes mellitus (T2DM)
  Lattuada G et al. Curr Diab Rep 2011;11:167 (narrative review)

- It is uncertain whether FL is a cardiovascular risk factor in T2DM patients
  Targher G at al. J Clin Endocrinol Metab 2013;98:483 (narrative review)

- It is even more uncertain whether FL increases the cardiovascular risk of individuals without T2DM
  Ghouri N et al. Hepatology 2010;52:1156 (narrative review)

How should I investigate?

- Define clearly and reproducibly your predictor of interest, i.e. FL
- Choose a study design controlling at least for known predictors
- Choose an “hard” cardiometabolic outcome

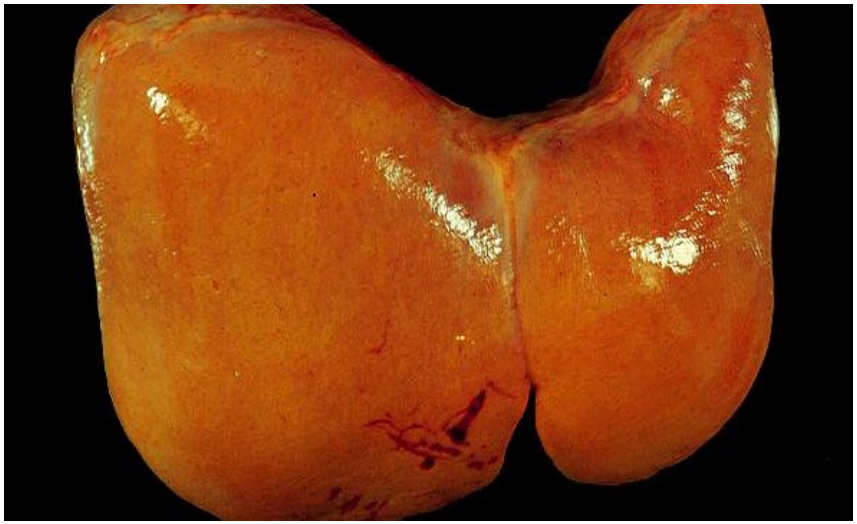
How should I investigate?

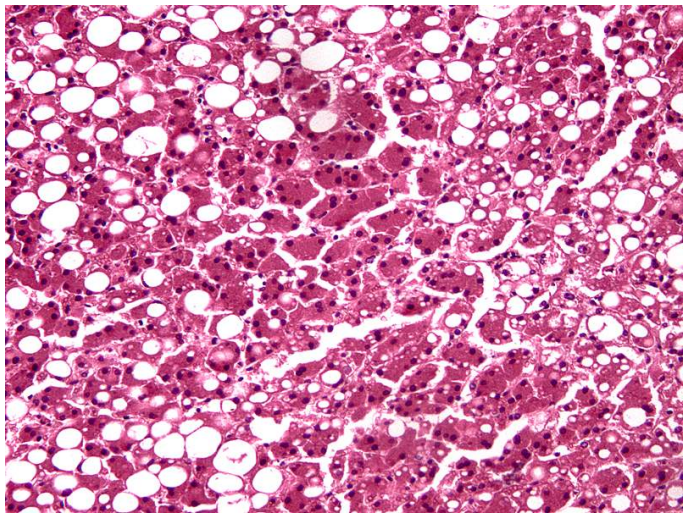
- *Define clearly and reproducibly your predictor of interest, i.e. FL*

- Choose a study design controlling at least for known predictors

- Choose an “hard” cardiometabolic outcome

An intuitive definition differs from…





… an operational definition

- How should I measure liver fat content?
- How should I rule out different causes of FL?

An operational definition

- *How should I measure liver fat content?*
- How should I rule out different causes of FL?

How should I measure liver fat?
4 competing methods

- Ultrasonography (US)
  - Ordinal measure: none < mild < moderate < severe
- Magnetic resonance spectroscopy (MRS)
  - Continuous measure
- Liver biopsy
  - Ordinal measure (current practice) – for tertiary care only!
- Surrogate indexes
  - Discrete or continuous measures calibrated against one of the above methods

Chalasani N. Am J Gastroenterol 2012;107:811 (AASLD guideline)
Vajro P. J Pediatr Gastroenterol Nutr 2012;54:700 (ESPGHAN guideline)
Ratziu V. J Hepatol 2010;53:372 (EASL guideline)

How should I measure liver fat?
The general population

- MRS – e.g. Dallas Heart Study
  Browning JD et al. Hepatology 2004;40:1387

- US – e.g. Dionysos Nutrition & Liver Study
  Bedogni G et al. Hepatology 2005;42:44

- Fatty liver index (FLI) – e.g. NHANES 2003/6
  Gerber L et al. Aliment Pharmacol Ther 2012;36:781
  Bedogni G et al. BMC Gastroenterol 2006;6:33 (FLI development)

How should I measure liver fat?
Between-method agreement

- Different methods do not necessarily agree but you could (try to) cross-calibrate them

- US compares well with liver biopsy for the prediction of moderate-severe degrees of FL

  Hernaez R. Hepatology 2011;54:1082 (systematic review)
  Shannon A. JPGN 2011;53:190 (Bambino Gesù Children's Hospital, Italy)

How should I measure liver fat?
Fat is not enough

- "As in other chronic liver diseases sampling variability is a limitation of liver biopsy in non-alcoholic fatty liver disease (NAFLD). Inflammatory lesions and ballooning are highly prone to sampling error as are fibrosis and steatosis which can result in misdiagnosis or understaging"

Ratziu V et al. J Hepatol. 2010;53:372 (EASL guideline)

See also Sanyal AJ et al. Hepatology 2011;54:344 (end-points for clinical trials)
See also Kleiner DE et al. Hepatology 2005;41 (histological scoring system)

How should I measure liver fat?
Fat is not enough

- Pure steatosis has a benign course as liver outcomes are concerned (general population and primary, secondary and tertiary care)

- Fibrosis is an "hard" hepatological outcome and non-alcoholic steatohepatitis (NASH) is a predictor of fibrosis (tertiary care)

Vernon G. Aliment Pharmacol Ther 2011;34:274 (systematic review)
Argo CK. J Hepatol. 2009;51:371 (systematic review)

How should I measure liver fat?
An error to avoid

- We can estimate the prevalence/incidence/evolution of NAFLD but not of NASH in the general population

- One should avoid inferring the number of patients with NASH in the general population from that of patients with NASH in tertiary care (different pre-test probability)

How should I measure other than liver fat? NASH and cardiometabolic disease

- The association between NASH and cardiometabolic risk factors is based mostly on cross-sectional studies (tertiary care)

Alisi A et al. Dig Liver Dis. 2011;43:143 (failing to replicating Brunt 2009)
Neuschwander-Tetri BA et al. Hepatology 2010;52:913 (adults)
Brunt EM et al. Hepatology 2009;49:809 (children)
Marchesini G et al. Hepatology 2003;37:917 (adults - milestone paper)

How should I measure liver fat?
The role of surrogate indexes

- Surrogate indexes are not intended to replace common diagnostic means in clinical practice

- Surely this was not our intention when we developed the fatty liver index (FLI) and tested the lipid accumulation product (LAP) as predictors of FL in the general population

Bedogni G et al. BMC Gastroenterol. 2006;6:33 (FLI development)
Bedogni G et al. BMC Gastroenterol. 2010;10:98 (LAP validation for FL)
Kahn HS. BMC Cardiovasc Disord. 2005;5:26 (LAP development)

How should I measure liver fat?
The fatty liver index (FLI)

- The FLI has gained much popularity as independent predictor of insulin resistance, diabetes (both prevalent and incident) and liver-related mortality (thanks Amalia for starting the world-wide interest in FLI!)

  Calori G et al. Hepatology 2011;54:145 (Cremona cohort study)
  Balkau B et al. BMC Gastroenterol 2010;10:56 (DESIR cohort study)
  Gastaldelli A et al. Hepatology 2009;49:1537 (RISC cross-sectional study)

- Does this imply that FL is an independent predictor of such outcomes? No.

How should I measure liver fat?
The fatty liver index (FLI)

Table 2

The parameters of the fatty liver index (FLI).

	β	SE (β)	STD (β)	*p*
Log_e (triglycerides, mg*dL^{-1})	0.953	0.211	0.308	<0.0001
BMI (kg*m$^{2-1}$)	0.139	0.050	0.353	0.006
Log_e (GGT, U*L^{-1})	0.718	0.202	0.278	<0.0001
Waist circumference (cm)	0.053	0.019	0.356	0.005
Constant	-15.745	1.631	-	<0.0001

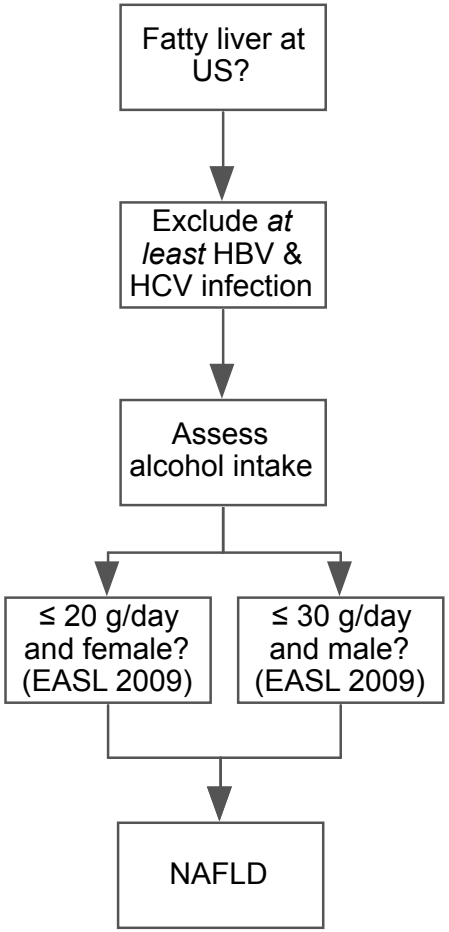
Abbreviations: β = regression coefficient; SE = standard error; STD = standardized value; loge = nathural logarithm. Other abbreviations as in Table 1. FLI is calculated by multiplying the predicted probabilities per 100 (see text for the formula).

Bedogni *et al. BMC Gastroenterology* 2006 **6**:33 doi:10.1186/1471-230X-6-33

An operational definition

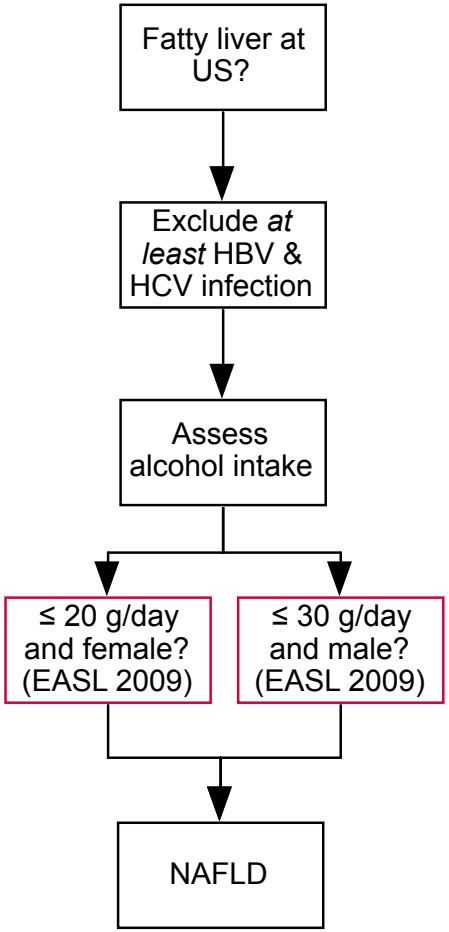
- How should I measure liver fat content?
- *How should I rule out different causes of FL?*

How should I rule out different causes? The common diagnostic algorithm



Ratziu V. *J Hepatol.* 2010;53:372 (EASL).

How should I rule out different causes?
Cut-points for ethanol intake



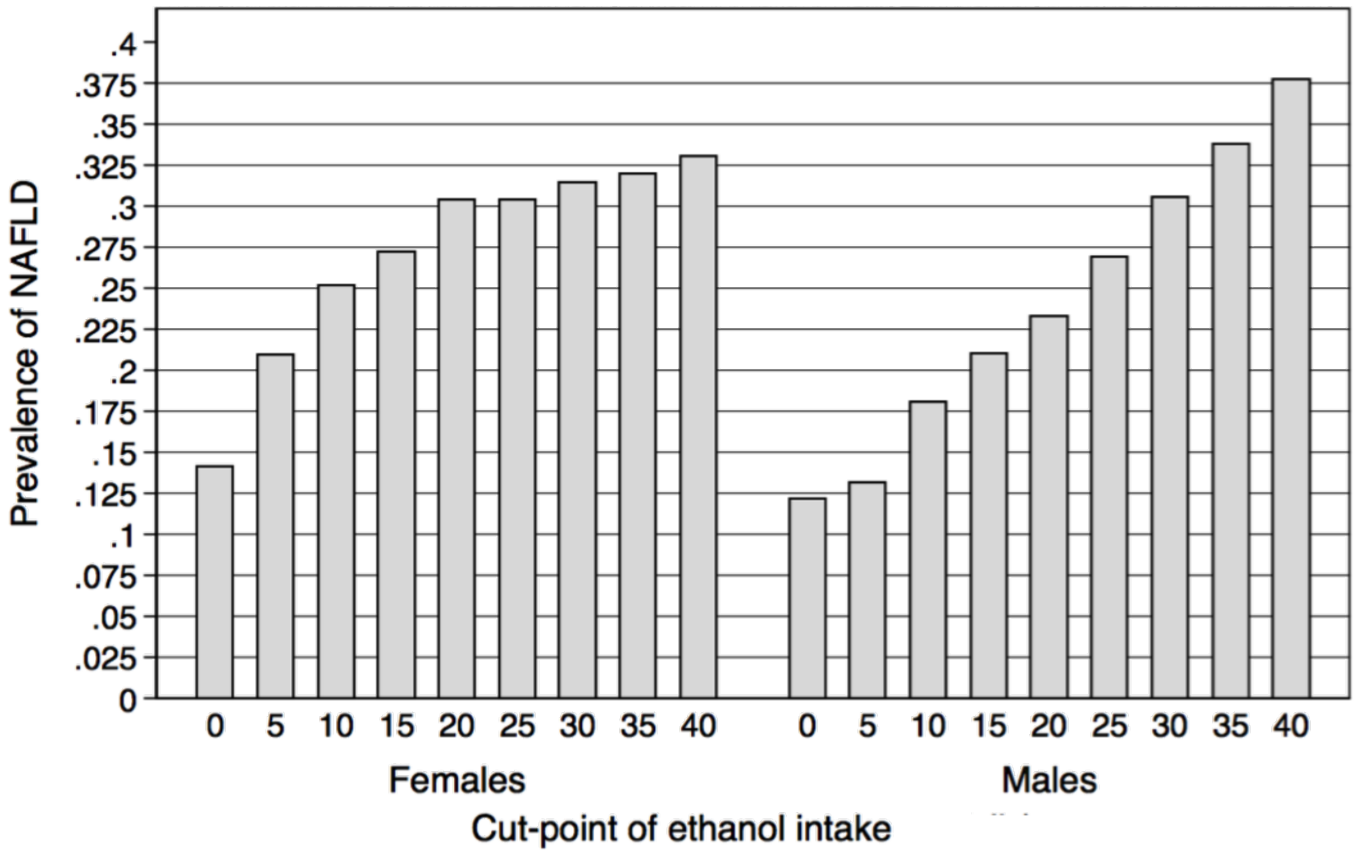
How should I rule out different causes?
Accuracy of ethanol measurement

- How accurate is my instrument to assess ethanol intake?

  MacDonald I *Health issues related to alcohol consumption.* Blackwell, 1999.

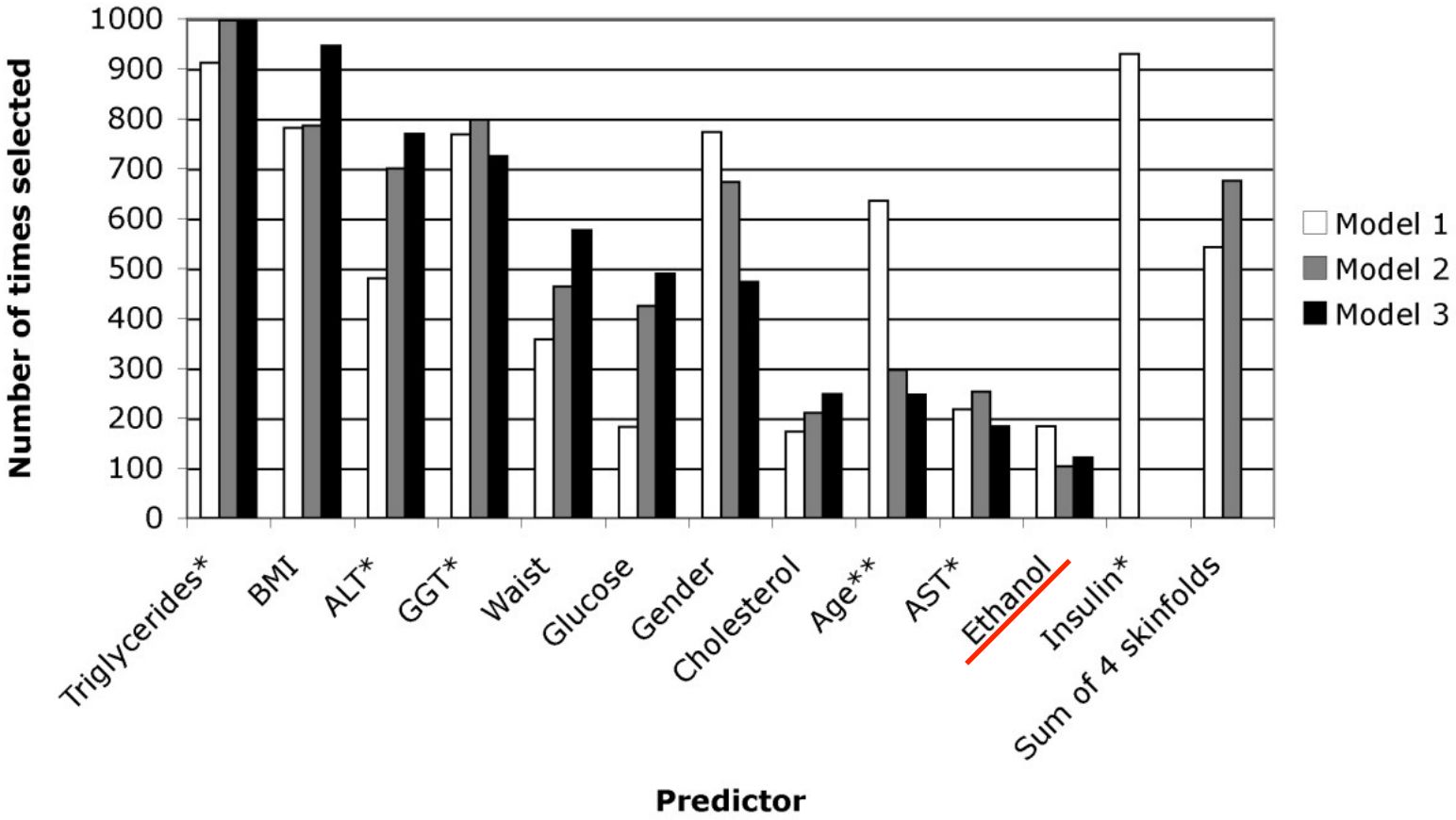
- This is what happens in the Dionysos Nutrition & Liver Study, which employed a 7-day-diary administered and collected by 2 research dietitians: see next slide.

How should I rule out different causes?
Changing the cut-point in the Dionysos Study



Calculated from Bedogni G. Hepatology. 2005;42:44

How should I rule out different causes?
A possible way out



Selection of candidate predictors at bootstrapped stepwise logistic regression. Bars indicate the number of times out of 1000 that the variables were selected for inclusion in 3 models. Model 1 is the starting model, Model 2 removes insulin and Model 3 removes skinfolds. Data are sorted using Model 3. Abbreviations: * = transformed using natural logarithm; ** = transformed using Box-Tidwell transformation (see text for details); other abbreviations as in Table 1.

Bedogni *et al. BMC Gastroenterology* 2006 **6**:33 doi:10.1186/1471-230X-6-33

How should I rule out different causes?
A possible way out

Table 4. Predictors of Fatty Liver Remission and Death in the Cohort With Fatty Liver

	FL Remission With SLD Remission (n = 70)		FL Remission With SLD Persistence (n = 85)		Death (n = 29)	
	RR (95% CI)	*P* Value	RR (95% CI)	*P* Value	RR (95% CI)	*P* Value
Male sex	0.67 (0.42-1.08)	0.100	1.02 (0.65-1.58)	0.935	6.05 (0.62-58.9)	0.121
Age (years/10)	1.15 (0.93-1.42)	0.201	0.97 (0.82-1.15)	0.734	2.78 (1.83-4.22)	<0.0001
BMI (kg/m^2)	0.99 (0.94-1.04)	0.663	0.95 (0.89-0.99)	0.043	0.95 (0.87-1.04)	0.266
Ethanol (g/day /20)	0.90 (0.81-.99)	0.043	0.81 (0.71-0.93)	0.002	1.10 (1.02-1.17)	0.005
ALT (U/L/40)	1.08 (0.71-1.66)	0.711	0.83 (0.52-1.34)	0.450	0.74 (0.34-1.62)	0.458
AST (U/L/37)	1.07 (0.62-1.83)	0.811	0.86 (0.40-1.84)	0.693	1.05 (0.31-3.57)	0.926
GGT (U/L/50)	0.95 (0.85-1.06)	0.335	0.92 (0.76-1.09)	0.323	1.21 (1.11-1.32)	<0.0001
Ethanol (g/day/20) × GGT (U/L/50)	–	–	–	–	0.99 (0.98-1.00)	0.077

RR = relative risk from Poisson regression with robust 95%CI

Bedogni G. *Hepatology.* 2007;46:1387 (Incidence and natural course of fatty liver in the Dionysos Study)

How should I rule out different causes?
History of ethanol intake

- Shouldn't I consider life-time alcohol consumption when evaluating the separate contribution of alcohol and other factors to FL?

- (I am not saying that this is an easy task…)

Bellentani S. *Hepatology.* 1994;20:1442 (first report of the Dionysos Study).

How should I measure liver fat?

- Define clearly and reproducibly your predictor of interest, i.e. fatty liver
- *Choose a study design controlling at least for known predictors*
- Choose an “hard” cardiometabolic outcome

Study design
2 study designs are better

- Observational study: a cohort study controlling for known confounders is the best option.
  - Is the change in liver fat associated with changes in the outcome of interest after correction for known confouders? e.g. Bedogni G et al. *Hepatology.* 2007;46:1387-1391.
- Randomized controlled trial: often unfeasible but can control for unknown risk factors (at least at baseline...)
  - e.g. does the change of fatty liver after a given treatment change the occurrence of the outcome of interest? Unanswered as yet for hard clinical outcomes.

Study design
Designs behind presently available evidence

- Most of the available evidence is based on cross-sectional studies

- Few “true” case-control studies are available

- Cohort studies of acceptable quality are presently available only for the FL-T2DM association

How should I investigate?

- Define clearly and reproducibly your predictor of interest, i.e. fatty liver
- Choose a study design controlling at least for known predictors
- *Choose an "hard" cardiometabolic outcome*

How should we investigate?
Try hard

- A “surrogate marker” may be fine as much as it has been associated with an hard clinical outcome (but many problems are lurking behind…)

- In the end, we will need to show that FL is independently associated with incident “hard” cardiometabolic outcomes, e.g. cardiovascular death

Conclusion

- Studying the FL-cardiometabolic disease association is fraught with methodological problems

- I have tried to point out the most relevant problems (or, if you prefer, those that have driven me insane in the last decade...)

Conclusion

- The way the FL-cardiometabolic disease will be studied in the next years will be central if we want to answer with greater confidence the question whether FL contributes to cardiometabolic disease

Thank you!