# The ceramide- and phosphatidylcholine- based Coronary Event Risk Test 2 (CERT2) and cardiovascular mortality in men and women with type 2 diabetes

Andreas Leiherer[1,3,4], Axel Muendlein[1], Christoph Saely[1,2,3], Barbara Larcher[1,2], Arthur Mader[1,2], Maximilian Maechler[1,2,3], Lukas Sprenger[1,2,3], Beatrix Mutschlechner[1,2,3], Magdalena Benda[1,2,3], Reijo Laaksonen[7,8], Mitja Laaperi[8], Antti Jylha[8], Peter Fraunberger[3,4], and Heinz Drexel[1,3,5,6]

[1]Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), Feldkirch, Austria; [2]Department of Medicine I, Academic Teaching Hospital Feldkirch, Feldkirch, Austria; [3]Private University of the Principality of Liechtenstein, Triesen, Principality of Liechtenstein; [4]Medical Central Laboratories, Feldkirch, Austria; [5]Department of Internal Medicine, Academic Teaching Hospital Bregenz, Bregenz, Austria; [6]Drexel University College of Medicine, Philadelphia, USA; [7]University Hospital, Tampere, Finland; [8]Zora Biosciences, Espoo, Finland

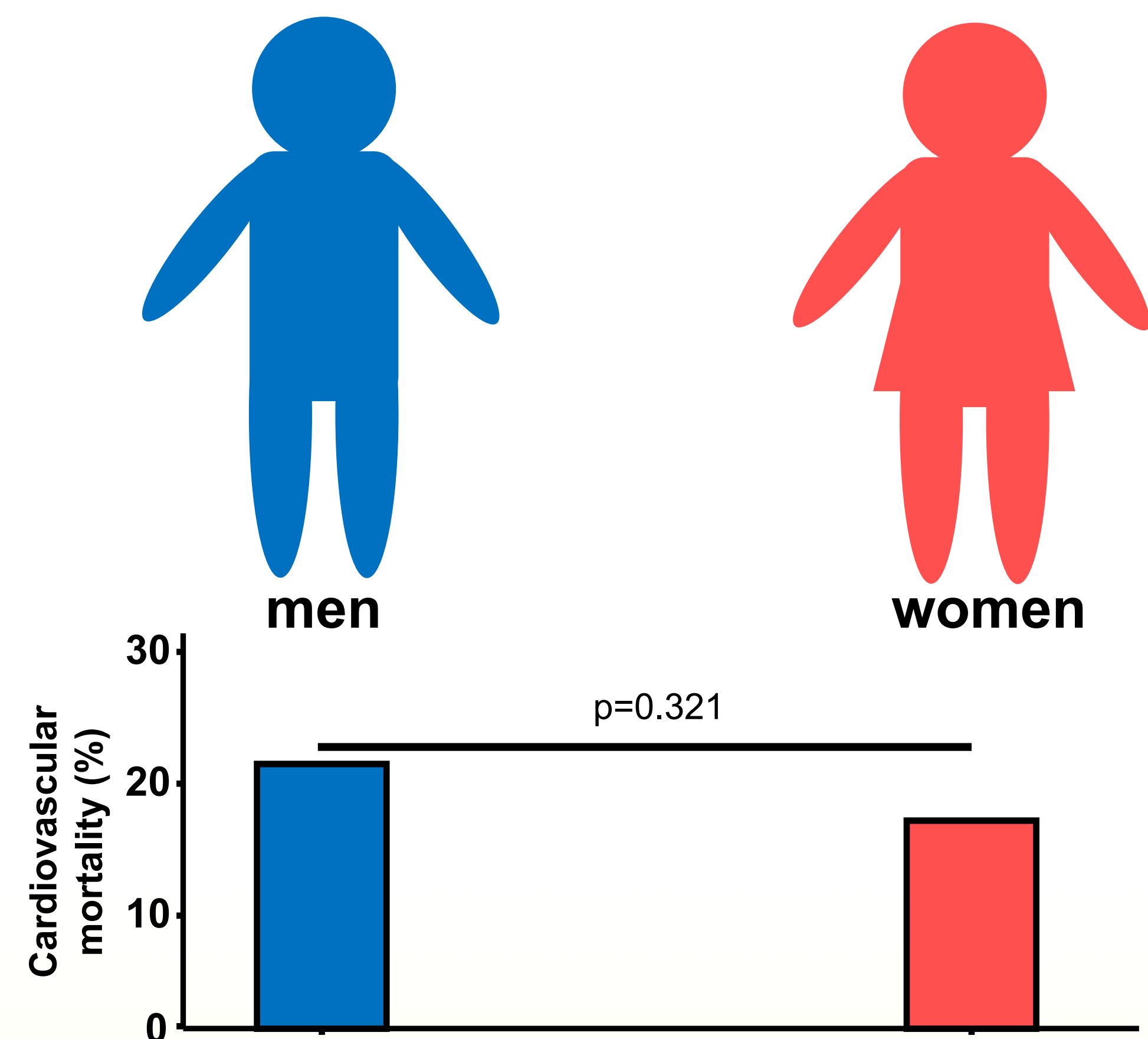All authors disclosed no conflict of interest

vivit@lkhf.at

## Background & Aim

The recently introduced Coronary Event Risk Test version 2 (CERT2) is a validated cardiovascular risk predictor score that uses circulating ceramide and phosphatidylcholine concentrations. We here aimed at investigating the power of CERT2 to predict cardiovascular mortality in men and women with type 2 diabetes (T2D).

## Patients & Methods

➤ **Patients:** 401 Austrian patients: 280 male and 121 female; all with T2D

➤ **T2D:** according to ADA criteria

➤ **CERT2:** coupled LC-MS/MS

➤ **Outcome:** cardiovascular (cv) death

➤ **Follow up:** 13 years

## What is CERT/CERT2

CERT – Coronary Event Risk Test is a blood based diagnostic test used to predict the risk of a cardiovascular event. Only ceramides and phosphatidylcholines are measured for this test.

The increased risk for fatal cardiovascular events may not be apparent in routine patient evaluations. It is important to note that more than half of patients hospitalized with a heart attack have normal LDL-C levels. Thus, high risk coronary artery disease (CAD) patients may have similar or even lower LDL-C concentrations than patients with more favorable prognosis. CAD may not even cause any apparent symptoms and the first symptom can be cardiovascular death.

# We conclude that gender does not significantly impact the association of CERT2 with cardiovascular mortality in patients with T2D.
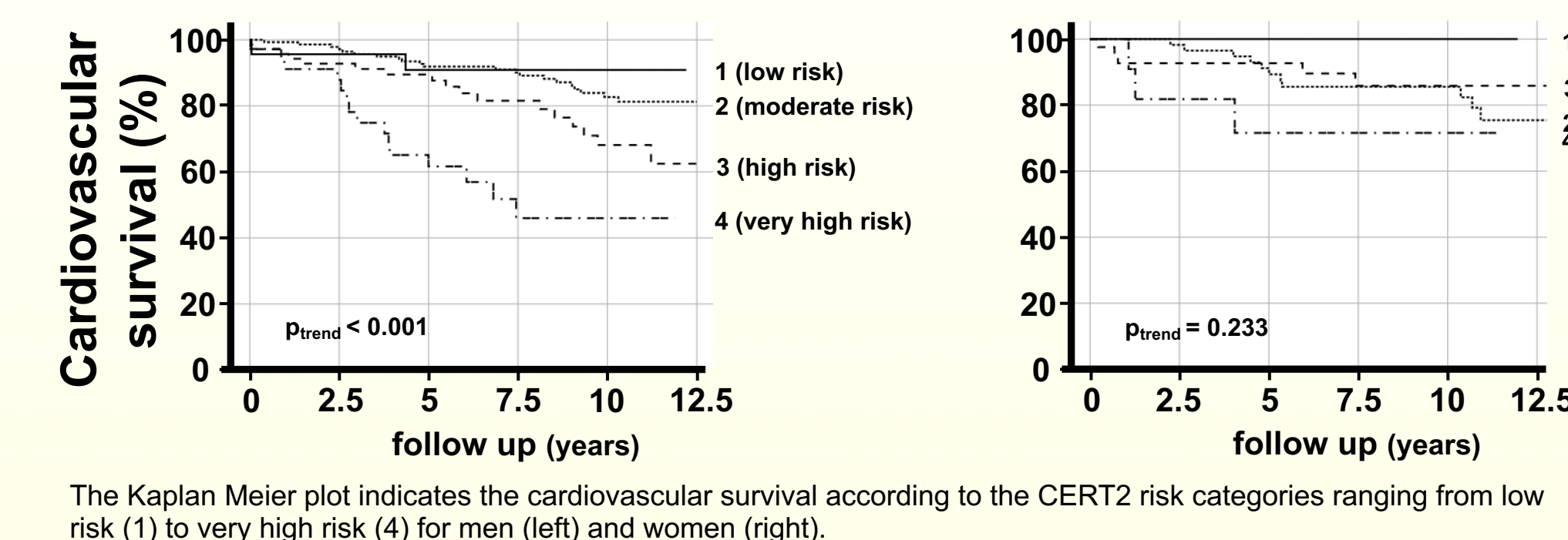
**men** **women**

### Patients´ Characteristics

| | men (n=280) | women (n=121) | p-value |
|---|---|---|---|
| Age (years) | 65.7 | 69.4 | 0.001* |
| BMI (kg/m$^2$) | 28.5 | 29.8 | 0.075 |
| LDL-C (mmol/L) | 2.9 | 2.9 | 0.966 |
| HDL-C (mmol/L) | 1.3 | 1.4 | <0.001* |
| Hypertension (%) | 93 | 97 | 0.137 |
| Smoking (%), current | 22 | 14 | 0.054 |
| Statin intake (%) | 63 | 66 | 0.490 |

All data are given as mean; p-values are derived from Mann –Whitney-U test and Chi-squared test, respectively; *denotes statistical significant difference
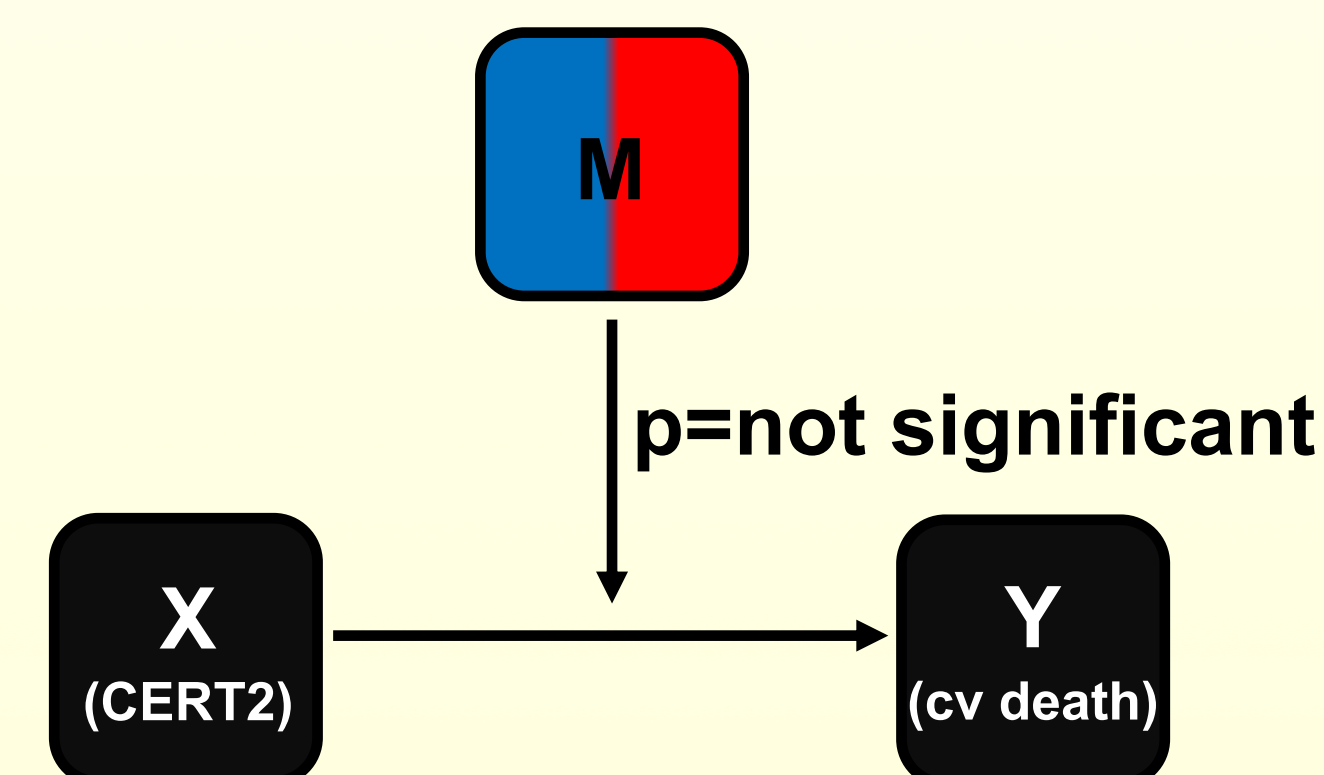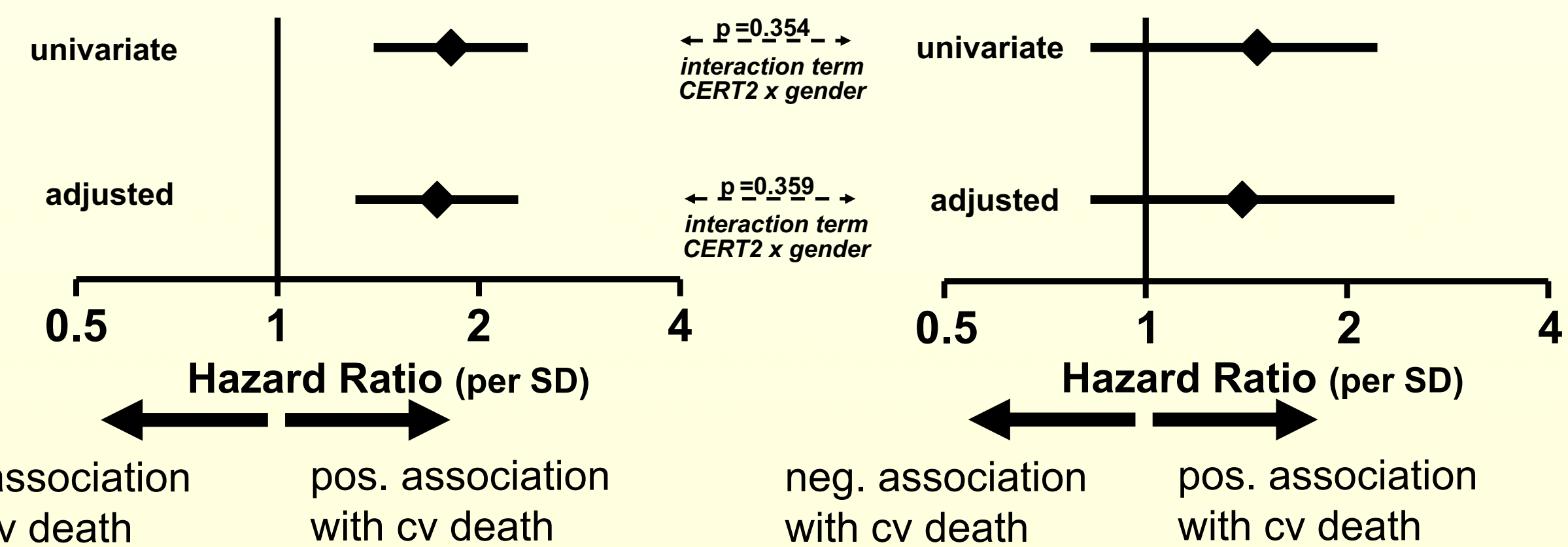
### Moderator analysis

M

p=not significant

X (CERT2) → Y (cv death)

Moderator analysis for the association between ceramide-based score CERT2 ands the outcome cardiovascular death with the moderator gender: The moderator gender was included in a regression model with the outcome cv death and the independent predictor CERT2. P-value (p=0.305) was calculated for the interaction of the independent variable and the moderator in the logistic regression model according to Hayes.

**Cardiovascular mortality of male and female T2D patients**

p=0.321

**Cardiovascular survival of T2D patients according to CERT2 categories**

1 (low risk)
2 (moderate risk)
3 (high risk)
4 (very high risk)

P$_{trend}$ < 0.001

P$_{trend}$ = 0.233

The Kaplan Meier plot indicates the cardiovascular survival according to the CERT2 risk categories ranging from low risk (1) to very high risk (4) for men (left) and women (right).

**Association of CERT2 score with cardiovascular mortality**

univariate — p =0.354, interaction term CERT2 x gender — univariate

adjusted — p =0.359, interaction term CERT2 x gender — adjusted

Hazard Ratio (per SD)

neg. association with cv death ← → pos. association with cv death

neg. association with cv death ← → pos. association with cv death

Association of risk predicting score CERT2 with cardiovascular mortality and overall mortality univariately and after multivariate adjustment: Forest plots represent hazard ratios per standard deviation with 95% confidence interval of Cox regression analyses. Model 1 represents univariate analyses. Model 2 adjusts for variables used for age, BMI, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol HDL-C, hypertension, current smoking, and statin intake. Interaction terms CERT2 x gender were non-significant both in univariate analysis (p=0.354) and after multivariate adjustment (p=0.359).

## What are ceramides?

**Ceramides (latin cera=wax) are wax-like lipid molecules. They are composed of a fatty acid and sphingosine.**

Fatty acid

Sphingosine
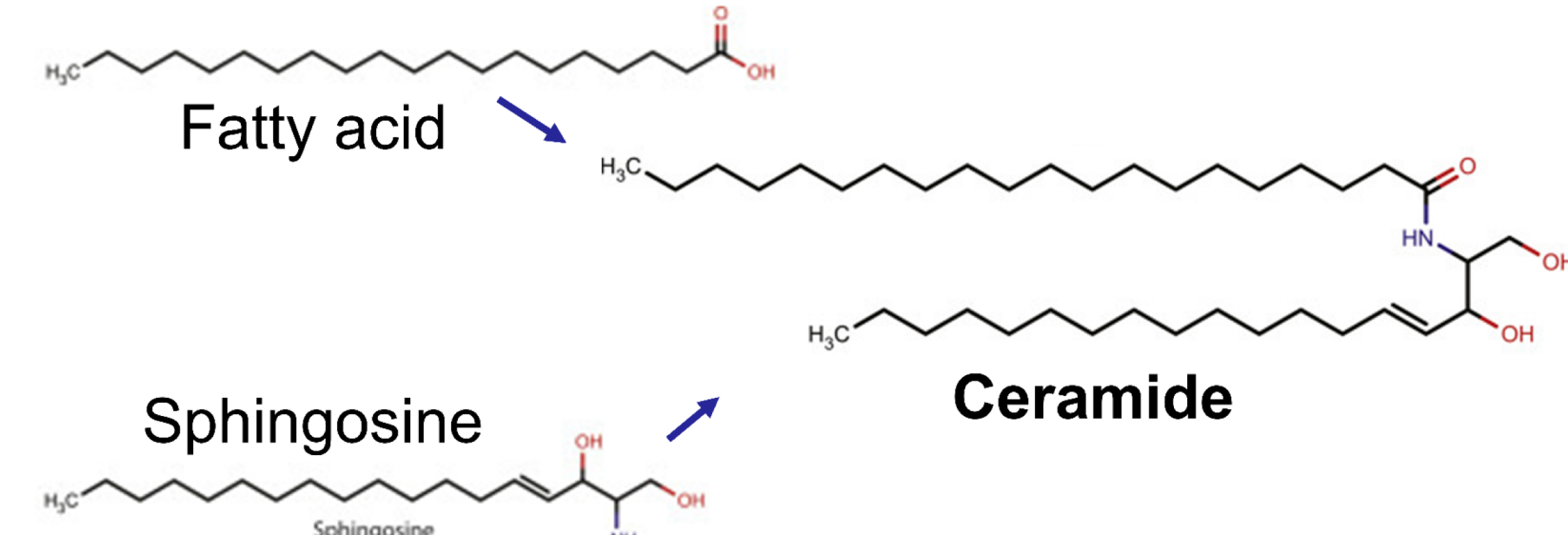
Ceramide

Ceramides have two major tasks: On the one hand, they are central components of the eucaryotic cell membrane (lipid bilayer). The incorporation of ceramides modulates the physical properties of the membrane (flexibility and function). On the other hand ceramides are also bioactive molecules and also free in blood. They carry out a wide range of molecular and physiological processes, for example endo- and exocytosis, proliferation, apoptosis, and inflammatory processes. They play also a role in the uptake of lipids into the endothelial cell and are associated with the formation of coronary plaques and are linked to diseases especially cardiovascular disease. Apart from ceramides, also phosphatidylcholines have a prognostic value for cardiovascular events and death. The combination of a certain set of phosphatidylcholines together with ceramides as this is done in CERT2 is a very potent in risk prediction

## How is CERT2 assessed?

**The CERT2 score and the respective CERT2 categories (1=low risk to 4=very high risk) are calculated based on ceramides, phosphatidyl-cholines, and their respective ratios.**

Circulating ceramides are measured by high-throughput LC-MS/MS assay. Recent studies reveal that circulating ceramide levels correlate strongly with future adverse cardiovascular events such as myocardial infarction and stroke. These studies have also created a foundation for relating ceramides to diabetes and heart disease. It is believed that ceramide testing become "new cholesterol" in clinical chemistry.
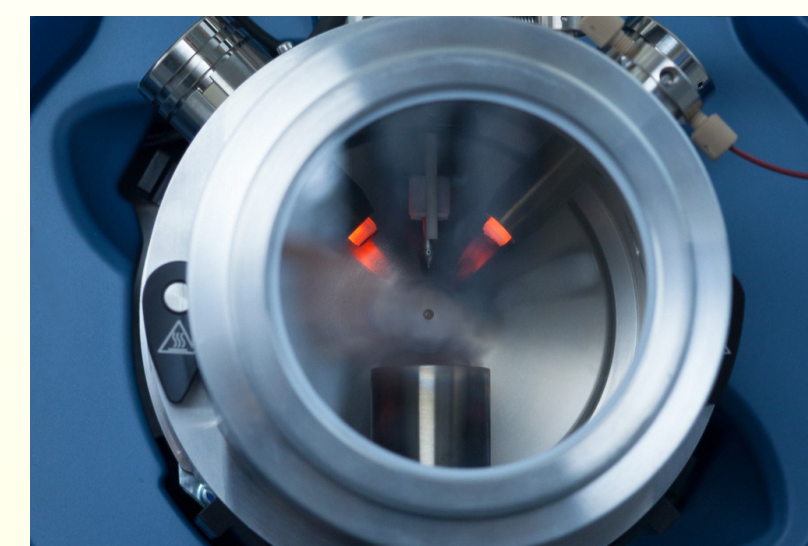
## Abstract

The recently introduced Coronary Event Risk Test version 2 (CERT2) is a validated cardiovascular risk predictor score that uses circulating ceramide and phosphatidylcholine concentrations. We here aimed at investigating the power of CERT2 to predict cardiovascular mortality in 280 male and 121 female patients with type 2 diabetes (T2D). Prospectively, we recorded 55 cardiovascular deaths in men and 19 in women during a mean follow-up time of 7.6±3.6 and 8.1±3.4 years respectively. Overall, cardiovascular survival decreased with increasing CERT2 risk categories (main figure poster), and cardiovascular mortality was higher in men than in women (20.1% vs. 15.8%; p=0.321). In Cox regression models, CERT2 significantly predicted the incidence of cardiovascular mortality in male patients with T2D (unadj. HR 1.82 [1.39-2.37] per standard deviation; p<0.001), the unadj. HR in women was 1.36 [0.83-2.22]; p=0.228). After adjustment for age, BMI, current smoking, LDL cholesterol, HDL cholesterol, hypertension, and statin use the HR in men was 1.73 [1.31-2.29]; p<0.001) and 1.40 [083-2.36]; p=0.210 in women. Interaction terms CERT2 x gender were non-significant both in univariate analysis (p=0.354) and after multivariate adjustment (p=0.359). We conclude that sex does not significantly impact the association of CERT2 with cardiovascular mortality in patients with T2D.