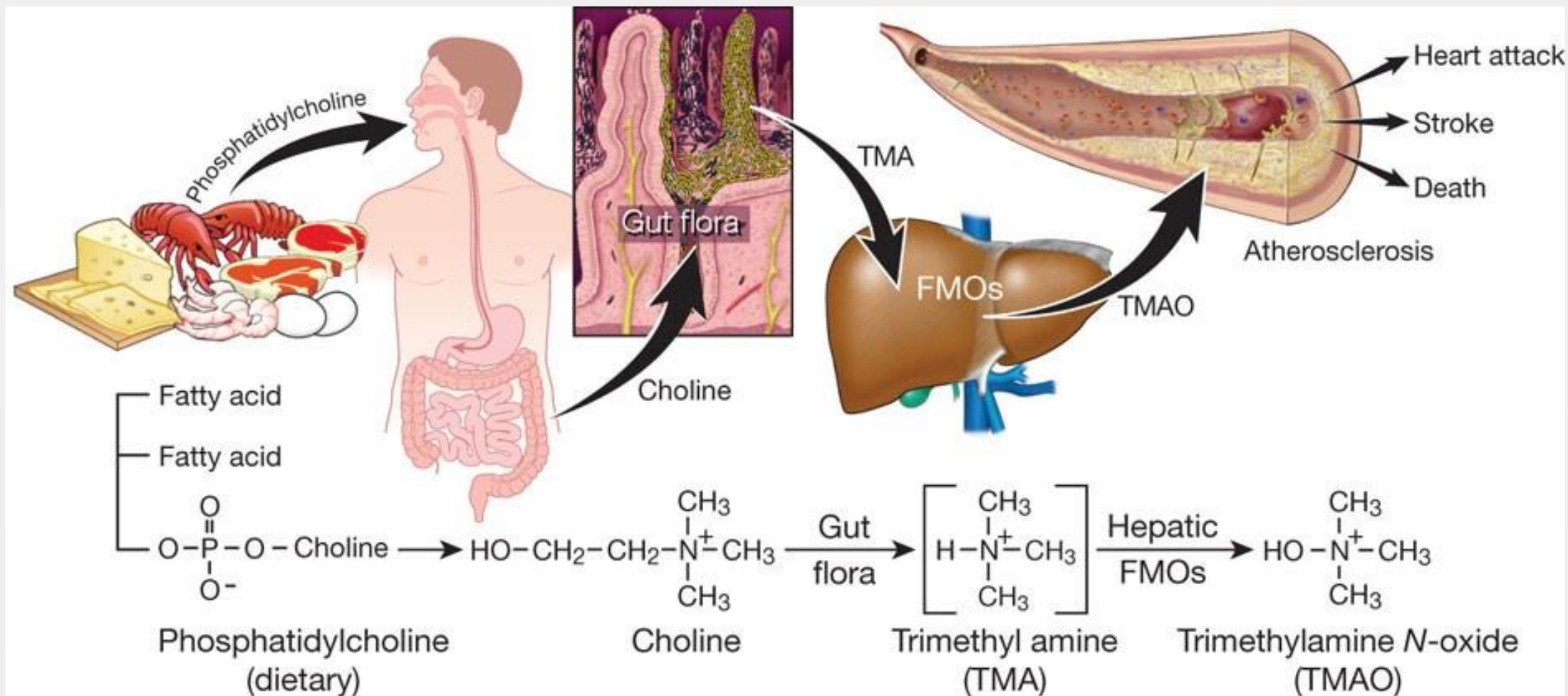# Personalized prediction of cardiac risk indicator *trimethylamine N-oxide* using artificial intelligence: a proof-of-concept study

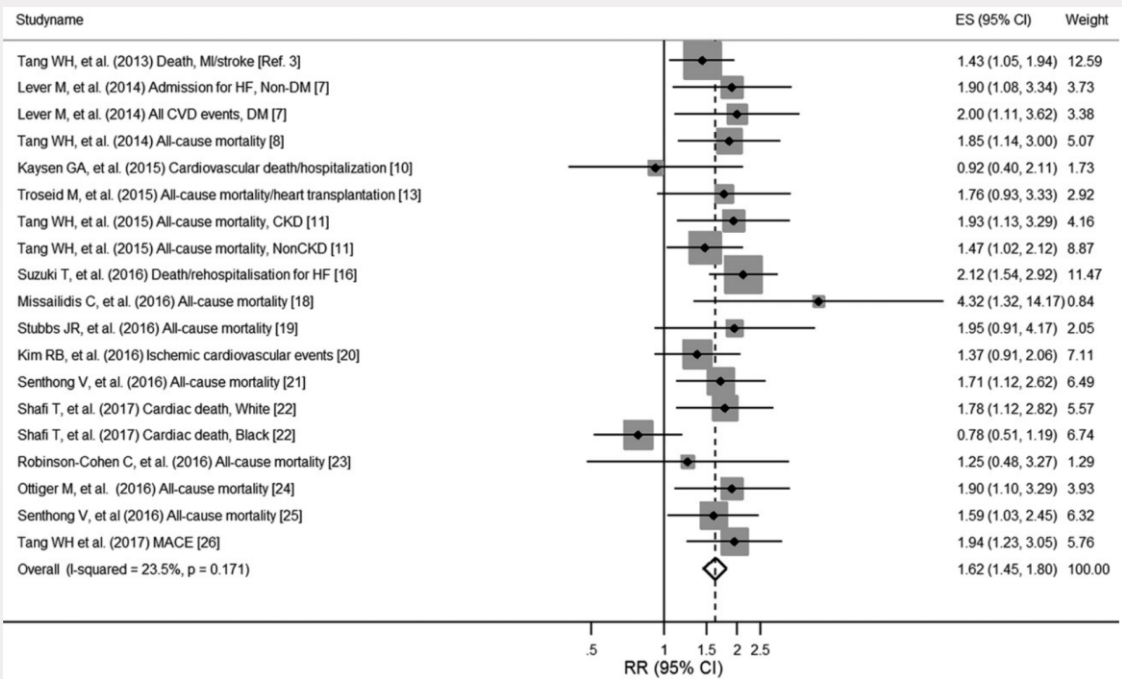**Siraphat Taesuwan, PhD**
Faculty of Agro-Industry
Chiang Mai University

Trimethylamine *N*-oxide (TMAO) is an emerging disease risk indicator

Wang, Z., Klipfell, E., Bennett, B. *et al. Nature* **472,** 57–63 (2011)

TMAO has prognostic values beyond traditional risk factors in cardiac patients

Strong TMAO—CVD association

Systematic review and meta-analysis shows higher blood TMAO was associated with 62% higher risk of cardiovascular death (19 studies from 2013-17)

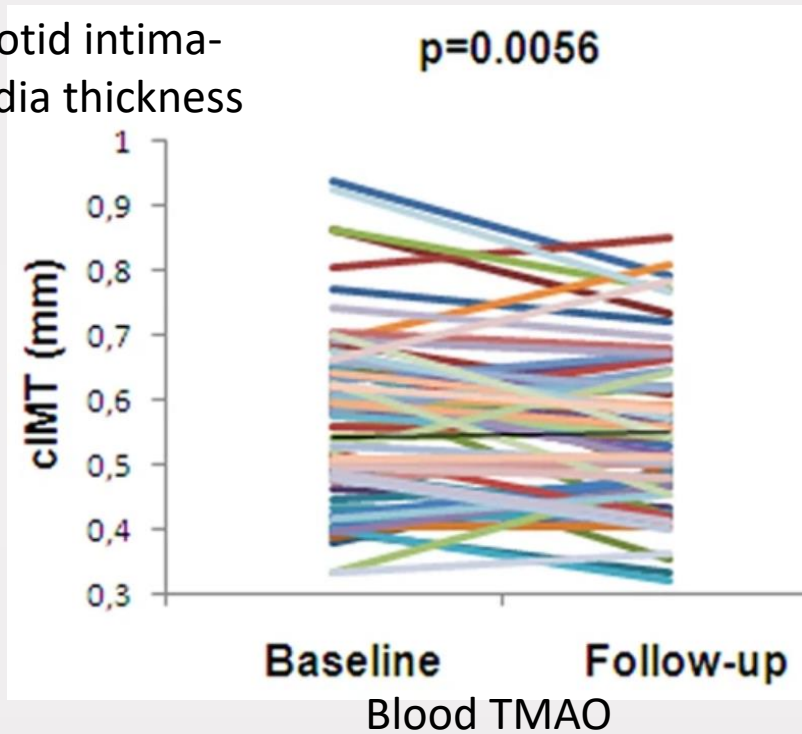Heianza Y, Ma W, Manson JE, Rexrode KM, Qi L. J Am Heart Assoc. 2017

TMAO levels predicted major adverse cardiac events as early as 30 days
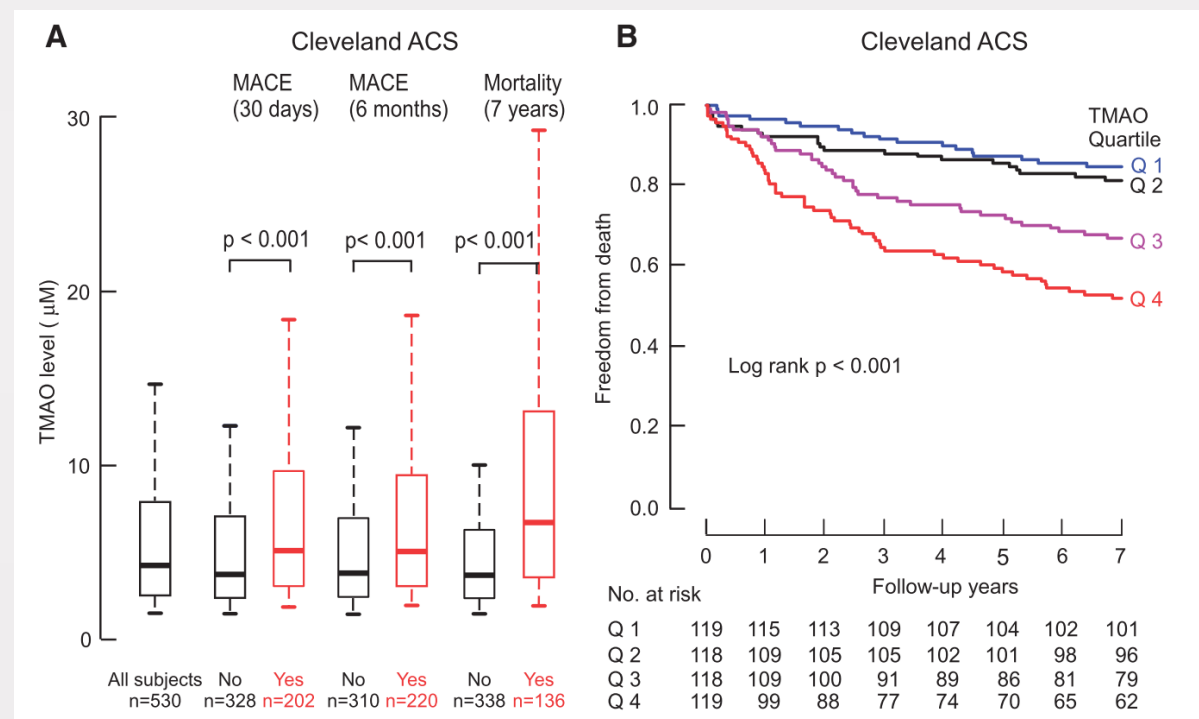

Li XS, Obeid S, Klingenberg R, Gencer B, Mach F, Räber L, Windecker S, Rodondi N, Nanchen D, Muller O, et al. Eur Heart J. 2017

Evidence suggests that TMAO is proatherogenic in overall healthy persons

Carotid intima-media thickness

Blood TMAO

Higher TMAO was associated increased cIMT in 220 at-risk individuals who were otherwise healthy

Randrianarisoa, E., Lehn-Stefan, A., Wang, X. et al. Sci Rep 6, 26745 (2016).
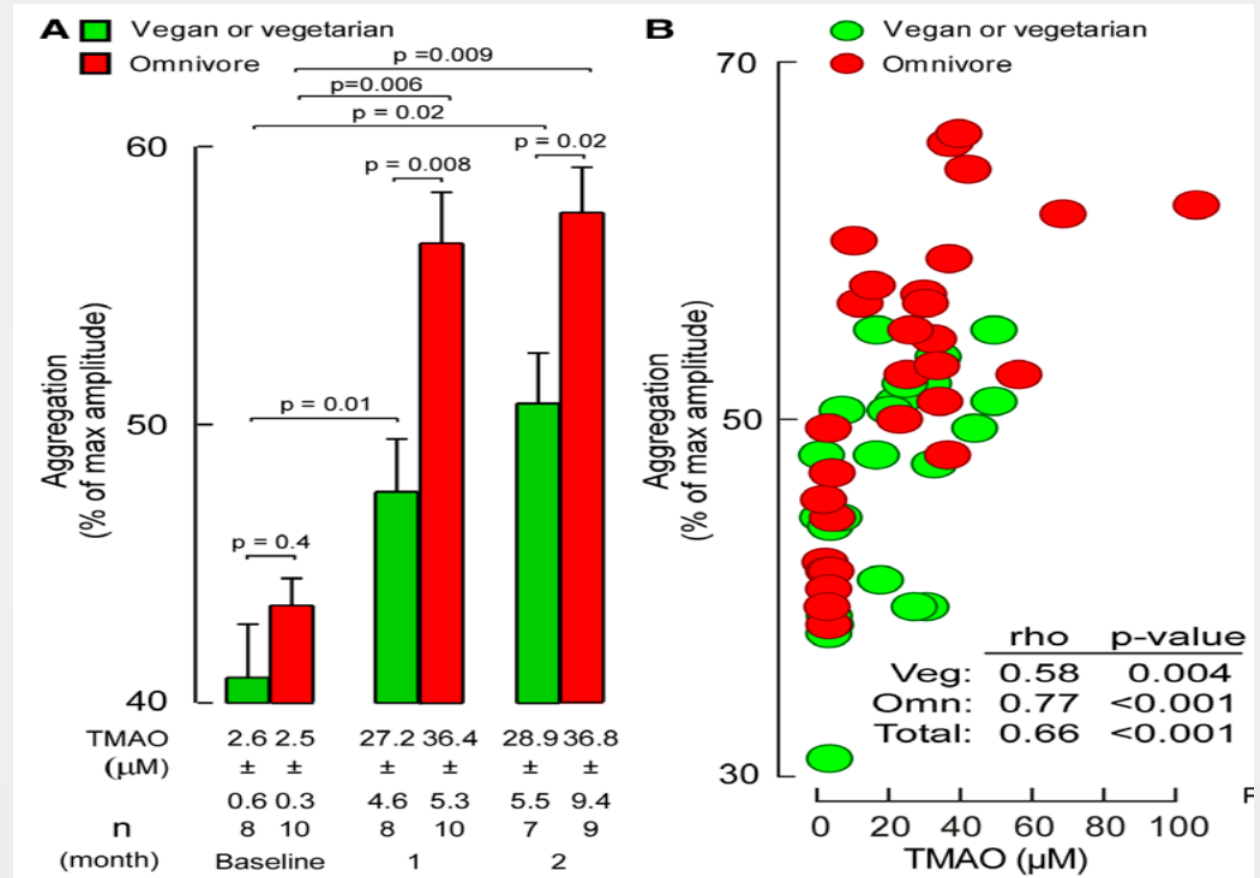
Healthy vegan/vegetarian (n=8) and omnivores (n=10) consumed 450 mg choline/day for 2 months exhibited increased TMAO levels associated with platelet aggregation
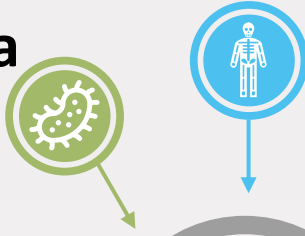
Zhu W, Wang Z, Tang WHW, Hazen SL. Circulation. 2017. p. 1671–3.

TMAO levels are a great target for *personalized* wellness strategies

- Age
- Estrogen
- Kidney function

Other factors

Gut microbiota

Genetic

Diet

Blood TMAO
(Trimethylamine *N*-oxide)

- Choline, carnitine ↑
- High fat↑ , low fat↓
- High protein↑ , low protein↓
- Indigestible carb↓
- Vegetarian↓
- Dairy ↑

Pathophysiologic changes

- Inflammation
- Oxidative stress
- Endothelial dysfunction
- Atherosclerosis
- Thrombosis

Chronic kidney disease

Colorectal cancer

Disease risks

Diabetes

Cardiovascular disease

- Predict major adverse cardiovascular events as early as 30 days in cardiac patients
- Predict 7-year mortality

Diet and lifestyle factors differentially influence TMAO levels (and disease risks) among people, presenting an opportunity to create a *personalized* health program

Conceptual framework

Personal and health parameters

MICROBIOME
16S rRNA gene sequencing

GENETIC
Flavin monooxygenase 3 G472A and A923G polymorphisms

QUESTIONNAIRES
Demography, semiquantitative Food Frequency Questionnaires, lifestyle (physical activity, eating and sleeping, smoking and defecation habits, alcohol consumption), medical/health status

ANTHROPOMETRICS & CARDIOVASCULAR INDICES
Weight, height, waist and hip circumference, %body fat, fat free mass, visceral fat, blood pressure and heart-rate, Carotid intima-media thickness (CIMT), Ankle Brachial Index Test (ABI), Cardio-ankle vascular index (CAVI)

BLOOD TEST
Fasting and postprandial TMAO, Complete blood count, fasting glucose, glycosylated hemoglobin (HbA1c%), liver aminotransferases activity (ALT, AST), total and HDL cholesterol levels, blood electrolytes, creatinine, etc.

Cross validation
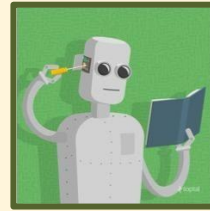
Machine learning

PERSONALIZED WELLNESS PLATFORM
Personalized lifestyle modification advice to keep TMAO and glucose in check

Prediction of risk factor levels

Prognostic values
- Fasting TMAO
- Fasting glucose

Inform intervention
- Postpradial TMAO response to a test meal

User: Health professionals and health-concious individuals

Drug target

TMAO monitoring devices

Recommended cut-off points for clinical care

Scope of the study (n=300; 100 from each country)
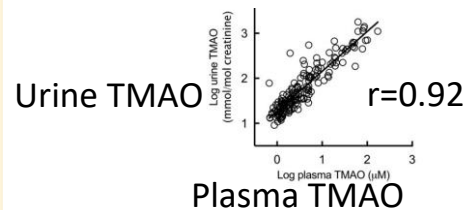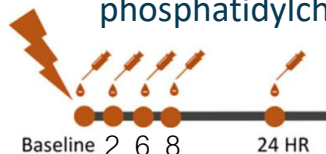
Implication

Study plan

PART I: PREPARATION

Step 1: Apply for Ethics approval

Step 2: Create study protocols

Pharmacokinetic study (n=15) to determine optimal sample collection time points

Oral choline challenge (500 mg phosphatidylcholine)

Baseline 2 6 8 24 HR

Urine TMAO   r=0.92

Plasma TMAO

TMAO method establishment

Development of data management platforms

Pilot testing of the final protocol

Recruit personnel

PART II: DATA COLLECTION

Step 1: Recruit participants

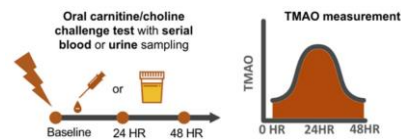**300 people**; overall healthy, no overt disease conditions

Sex: M,F; Age **30-70** years; Overweight or obese

Step 2: Screening

Step 3: Study visit

Questionnaires, anthropometry, a stool sample, fasting blood draws and urine

Choline challenge test (urine/blood)

Oral carnitine/choline challenge test with serial blood or urine sampling   TMAO measurement

Baseline 24 HR 48 HR   0 HR 24HR 48HR

PART III: LAB ANALYSIS

TMAO (LC-MS/MS); CBC & Blood chemistry analyzers; microbial DNAsequencing; SNP analysis (PCR)

PART IV: ALGORITHM DEVELOPMENT

Step 1: Data cleaning

Step 2: Machine learning algorthms

Prediction of fasting TMAO, fasting glucose, TMAO responses

PART V: PERSONALIZED WELLNESS PLATFORM

Personalized lifestyle modification advice to keep TMAO and glucose in check

Year 1-2   **Year 3**

Country-specific prediction algorithms

Collaborative network in nutrition

Personalized wellness plaform

Publication in an ISI/Scopus journal

Outputs

TMAO monitoring device

Genetic sequencing

Metabolomics

Personalized intervention

Possible extension