Phytochemicals, Metabolomics and Anti-diabetes

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Key to promote human health in aging societies: better understanding of major degenerative diseases

a. Better knowledge in genes and environmental interactions
b. Better disease treatment strategy: prevention
c. Better understanding in human ageing and healthy ageing

a. Atherosclerosis and related cardiovascular disease (CVD)
b. Diabetes: from insulin resistance and metabolic syndrome (pre-diabetes) to T2DM, and diabetic complications (CVD and DN)
c. Cancer
d. Alzheimer’s disease (AD)
Where West meets East

The concepts of Asia’s traditional medicines might sound alien to Western ears, but some of them are starting to evolve to fit scientific investigation.

BY PENG TIAN

Medicines and foods are isogenic

In 1578, Li Shizhen compiled The Compendium of Materia Medica, the most comprehensive documentation of the use of medicinal herbs, minerals and animal parts.
Why most clinical trials of TCM (or herbal medicines) fail?

a. The formula is not effective
b. The disease target is not suitable
c. The dosing is not right (should be higher)
d. The treatment stage is too late (and/or duration is not long enough)
Our working hypothesis and strategy: atherosclerosis as a disease target

(a) Regression of atherosclerosis may occur by treatment of key events in early pathogenesis.

(b) Herbal medicines, if effective, may exhibit therapeutic potential in early, reversible stage.

Strategy

> To combine enriched active herbal preparations to inhibit multiple key events in early pathogenesis of atherosclerosis.

> Is the use of compound formulae (fufang) in the right direction?
The linear sequence of events leading to early atherosclerosis

1. Hypercholesterolemia (high TC and LDL-C)
2. Monocyte adhesion to arterial endothelium and penetration of monocytes into the artery
3. Phenotypic modulation in activated macrophages, expression of scavenger receptors, and uptake of oxidized LDL
4. Fatty streak formation (cholesterol-rich foam cells)
5. Atherosclerotic plaques


Current working hypothesis of atherosclerosis

a. Low-density lipoprotein (LDL) oxidation
b. Endothelial dysfunction
c. Inflammation
The linear sequence of events leading to in early atherosclerosis

Glass and Witztum, Cell, 104, 503-516, 2001
Screening for phytochemicals and crude extracts to inhibit key events

A. Antioxidants to inhibit low-density lipoprotein (LDL) oxidation: Inhibition of LDL oxidation triggered by Cu²⁺

B. Agents to inhibit endothelial dysfunction: Inhibition of selectins, ICAM, and VCAM in cultured human aortic endothelial cells (HAECs)

C. Agents to reduce endothelial damage: Inhibition of oxLDL-induced endothelial cell injury

D. Scavenger receptor inhibitors (SRI) to reduce foam cell formation: Inhibition of oxLDL uptake by cultured J774 macrophages
What can be the new herbal regimens? We have screened for >2,000 phytochemicals and crude extracts from medicinal plants

1. 血府逐瘀汤（清 王清任）
   柴胡、桔梗、枳椇、川牛膝、生地、赤芍、川芎、紅花、甘草、當歸、桃仁

2. 冠心二號方（大陸）
   丹參、赤芍、川芎、紅花、降香

3. 複方丹參滴丸（大陸）：丹參、三七、冰片

4. 芎芍膠囊（大陸）：赤芍、川芎

New formula (VGH-SAGE-1): What is it?
Table 1. (a) Composition of human LDL and (b) oxidation of PUFAs in LDL

(A) Composition of human LDL

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Apolipoprotein B-100</td>
<td>1</td>
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<tr>
<td>Phospholipids (PL)</td>
<td>800-900</td>
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<tr>
<td>Cholesterol</td>
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</tr>
<tr>
<td>Esterified</td>
<td>1,500-1,600</td>
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<tr>
<td>Unesterified</td>
<td>500-600</td>
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<tr>
<td>Triacylglycerol (TG)</td>
<td>150</td>
</tr>
<tr>
<td>(\alpha)-Tocopherol</td>
<td>6-12(^a)</td>
</tr>
<tr>
<td>(\gamma)-Tocopherol</td>
<td>0.5</td>
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<tr>
<td>(\beta)-carotene</td>
<td>0.1-0.4</td>
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<tr>
<td>Lycopene</td>
<td>0.2-0.7</td>
</tr>
<tr>
<td>Ubiquinone-10</td>
<td>0.5-0.8</td>
</tr>
<tr>
<td>Other lipophilic antioxidants</td>
<td>trace</td>
</tr>
</tbody>
</table>

\(^a\)Per LDL particle.

(B) Oxidation of PUFAs in LDL

A. Major polyunsaturated fatty acids (PUFA) in human LDL illustrating the presence of double allylic sites

\[
\begin{align*}
\text{Linoleic acid (18:2)} & : \quad \text{Arachidonic acid (20:4)} \\
\end{align*}
\]

B. Lipid peroxidation of polyunsaturated fatty acids (PUFA)

C. Reaction of malonaldehyde (MDA) with thiobarbituric acid (TBA)
In search of antioxidants to inhibit Cu$^{2+}$-induced LDL oxidation

- Prolong lag phase ($\Delta T_{\text{lag}}$)
- Reduce slope (RXN rate)
## Table 1. Lag phase and relative potency of Salvianolic acid B (Sal B) to inhibit LDL oxidation

<table>
<thead>
<tr>
<th></th>
<th>$T_{\text{lag}}$ (min)</th>
<th>$\Delta T_{\text{lag}}$ (min)</th>
<th>Relative Potency</th>
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<tr>
<td>Control</td>
<td>151</td>
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<tr>
<td>Probucol</td>
<td>302</td>
<td>151</td>
<td>1.00</td>
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<tr>
<td>Trolox</td>
<td>251</td>
<td>100</td>
<td>0.66</td>
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<tr>
<td>Quercetin</td>
<td>422</td>
<td>271</td>
<td>1.79</td>
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<tr>
<td>Genistein</td>
<td>216</td>
<td>65</td>
<td>0.43</td>
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<td>Sal B</td>
<td>1,286</td>
<td>1,135</td>
<td>7.52</td>
</tr>
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</table>

1. LDL (50 μg/mL LDL-C) oxidation was induced by 5.0 μM Cu$^{2+}$.
2. LDL oxidation was determined by conjugated diene formation. The increase of UV absorption at 234 nm was continuously monitored. Samples at 1.0 μM were assayed.
3. The potency of probucol (as a positive control) was set as 1.00.
4. Results are mean values of three determinations.

Phenolic phytochemicals in *Salvia miltiorrhiza*

Salvianolic acid A (Sal A)

Rosmarinic acid

Salvianolic acid (Sal B)

Caffeic acid

Protocatechuic acid

Lithospermic acid

Danshensu

*Salvia miltiorrhiza (Danshen)*
Phytochemicals and herbal extracts to inhibit endothelial damage

a. Inhibition of LDL oxidation (LDL oxidation triggered by Cu$^{2+}$)
b. Inhibition of selectins, ICAM, and VCAM in cultured human aortic endothelial cells (HAECs)
c. Inhibition of oxLDL-induced human aortic endothelial cell (HAECs) injury
d. Inhibition of oxLDL uptake by macrophages (J774 macrophages)
c. Inhibition of oxLDL-induced human aortic endothelial cell (HAECs) injury

1. Control
2. oxLDL (40 µg/mL)
3. oxLDL 40 (µg/mL) + SB (20 µM)
4. oxLDL (40 µg/mL) + SB (50 µM)
What have we found?

>>Inhibition of oxLDL-induced endothelial cell (HAECs) injury.

1. Agents that inhibit oxLDL-induced endothelial injury.

2. Agents that inhibit high glucose-induced HAEC injuries

3. Agents that stimulate endothelial cell proliferation (angiogenic agents)

4. Agents stimulate cell proliferation (ex. hepatocytes/hepatoma cells)

Figure 2. En face immunoconfocal images of aortic endothelium of apoE(-) mice. Upper row: animals with a normal plasma glucose level; Lower row: animals with hyperglycemia post streptozotocin (STZ) treatment. **Tremendous reduction of connexins 37 and 40 in STZ groups**. Yeh H.-Y. et al. Arterioscler Thromb Vasc Biol 23, 1391-1397, 2003.

**Protection of high-glucose induced endothelial injury**
1. Cultured murine macrophages (J774A.1) were chosen as the cellular model.

2. Uptake of oxLDL by J774A.1 macrophages was determined by Oil Red staining.

3. Helvolic acid (HA) was a positive control.
A: Control
B: Ox LDL
C: Ox LDL + HA
D: Ox LDL + VGH 801-W1
Scavenger Receptor Inhibitors: What are they?

Anionic organic acids with rigid structural skeletons

Helvolic acid (HVA)

Ganodermic acid S
SAGE: as a candidate of new herbal medicine

1. *Salvia miltiorrhiza* (Labiata): 丹參
2. *Angelica sinensis* (Umbelliferae): 當歸
3. *Glycyrrhiza uralensis* (Leguminosae): 甘草

One-step Extracts

Salvianolic acid B (Sal B): as a drug lead
Potential mechanisms of salvianolic acid B (Sal B) to reduce atherosclerosis

1. Sal B is a strong antioxidant to inhibit LDL oxidation *in vitro* and *ex vivo* (<1 µM)
2. Sal B protects (oxLDL-induced) endothelial dysfunction/injury in cultured human aortic endothelial cells (HAECs) (2.5-20 µM)
3. Sal B is a mild scavenger receptor inhibitor
4. Sal B is an anti-inflammatory agent. Sal B inhibits COX-2 expression
Evaluation of the anti-atherosclerotic effects

ApoE-deficient (ApoE(-)) mice: Spontaneous atherosclerosis due to elevated plasma cholesterol, impaired VLDL metabolism, and accelerated lipoprotein oxidation.

VLDL (apoB/apoE) → IDL → LDL (apoB)

Progression of atherosclerosis in apoE (-) mice

Fig. 1. Pathogenesis of atherosclerosis in apolipoprotein E-deficient mice fed with a western type diet or a chow diet.

(from Nakashima et al., 1994)
Animal Study on Sal B and SAGE ApoE(-) mice as an animal model

>>Male apoE(-) mice (19 weeks of age), fed with a chow diet containing 0.15% (w/w) cholesterol, were chosen as the animal model.

1. Control (normal diet plus 0.15% cholesterol)
2. Sal B (0.28%, w/w)
3. SAGE (2.0%, w/w)
4. Positive control (DPPD) (0.5%, w/w)

The duration of feeding experiment was 3 months.
Reduction of atherosclerosis by Sal B

C57/BL6J  apoE(-)  Sal B-treated apoE(-)

Fig. 1. Histopathological features of cross-sections of thoracic aortas from C57/BL6J, apoE(-), and Sal B-treated apoE(-) mice. *p < 0.05 when compared with the apoE(-) group. (Chen and Shiao)
Table 1. Reduction of atherosclerotic area in apoE(-) mice

<table>
<thead>
<tr>
<th></th>
<th>Control (n=14)</th>
<th>Sal B (n=14)</th>
<th>SAGE (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>16,291±9,737</td>
<td>12,096±3,314</td>
<td>12,409±2,774</td>
</tr>
<tr>
<td>Intima</td>
<td>9,586±6,201</td>
<td>2,345±2,927</td>
<td>1,863±1,873</td>
</tr>
<tr>
<td>Intima/Media</td>
<td>0.485±0.380</td>
<td>0.165±0.185</td>
<td>0.138±0.108</td>
</tr>
<tr>
<td>P value</td>
<td>0.0087</td>
<td>0.0029</td>
<td></td>
</tr>
<tr>
<td>Inhibition (%)</td>
<td>66</td>
<td>72</td>
<td></td>
</tr>
</tbody>
</table>

a. Unit for media and intimal area (10³ µm²)
b. Sal B: Salvinanolic acid B from *Salvia miltiorrhiza*
Diabetes: the key human degenerative disease
Diabetes in numbers

90 M Chinese live with diabetes and 1.3 M died in 2011.
New platform technology is required in diabetic research: can metabolomics help?

Interaction and contribution of multiple risk factors in the progression of obesity, insulin resistance (IR), metabolic syndrome (MS), type 2 diabetes mellitus (T2DM), diabetic nephropathy (DN), diabetes-accelerated atherosclerosis and CVD.
What is metabolomics (metabonomics)?

**Metabonomics** (Nicholson et al., 1999)

The quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification

**Metabolomics** (Fiehn, 2002)

A comprehensive and quantitative analysis of all metabolites in a system
Metabolomics (Metabonomics)

>>Global profiling and determination of low-molecular-weight (LMW) metabolites in normal and pathophysiological states.

a. Molecular fingerprinting/chemical phenotyping/chemical individuality

b. Information more dynamic and close to the functional end (phenome, Nicholson)

Estimated number of LMW metabolites In humans

a. Primary metabolites: ~3,000

b. Secondary metabolites: 3,000~? (gut microflora?)
Metabolomics: Untargeted vs. targeted metabolomics

Samples → Q-TOF → Data collection → Global (untargeted) metabolite profiling

Data analysis → Volcano plot → Heat map (ANOVA) → Condition tree (Clustering) → Principal component analysis (PCA) → Metabolome: Metabolic pathways and interaction

Targeted metabolite identification → Function and dysfunction

Cheng & Shiao
Two approaches

a. **Untargeted metabolomics**: global metabolite profiling (without knowing the metabolite identity and absolute concentration) in order to search for potential metabolites that may differentiate into groups.

   *(hypothesis-generating)*

b. **Targeted metabolomics**: Identification and quantitation of potential metabolites that contribute to grouping. This approach is most suitable for clinical studies.

   *(hypothesis-based, mission-oriented)*
What metabolomics can do?

1. To better diagnose disease or predict the risk of disease and disease progressing
2. To determine whether a treatment is effective
3. To speed the new drug development and to make safer drugs (herbal medicine)
4. To monitor healthy people for early signs of diseases (studies on healthy ageing)
Integration of OMICS

a. Genome-wide association studies (GWAS) have identified many risk loci for complex diseases, but effect sizes are typically small.

b. Associations with **metabolic traits** as functional intermediates can overcome these problems and potentially inform **individualized therapy**.

>A comprehensive analysis of genotype-dependent metabolic phenotypes using a **GWAS** with non-targeted metabolomics.

Our studies

a. Animal studies: High-fructose (HF) and high-lipid (HL) diet fed and STZ-treated (DM) male SD rats

b. Clinical study to search for pre-diabetes biomarkers:
   A community cohort study
   >>HPLC-TOF MS based untargeted (global) metabolomics approaches to search for portfolio of biomarker in urine and plasma reflecting features of metabolic syndrome as a pre-diabetic state

c. Clinical study: A healthy, aged community cohort study
   >>600 MHz FT-NMR based targeted metabolomics study of urine and plasma metabolomes
Animal study: Male SD rats


CT: Control diet
HF: High-fructose diet
HL: High-lipid diet
DM: STZ + control diet
QC: urine-mixed samples

High fructose diet (HF)   High-lipid diet (HL)   QC: Mixed samples

Figure 8. The Principal Component Analysis (PCA) plot of urine samples.

CT, rats fed with a control diet; HF, rats fed with a high-fructose diet; HL, rats fed with a high-lipid diet; DM, rats induced diabetes by a single intraperitoneal injection of streptozotocin (65 mg/kg BW) and fed with a control diet, QC, urine mix standard. The PCA plot was shown in color yellow (QC), black (CT), green (HF), red (HL), blue (DM), which were shown on the right.
<table>
<thead>
<tr>
<th>Pathway</th>
<th>Metabolites tentatively identified</th>
<th>HF</th>
<th>HL</th>
<th>DM</th>
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<td>Indoxyl</td>
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<td>3-Hydroxyanthranilic acid</td>
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<td>↓</td>
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<td>Formylantranilic acid</td>
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<tr>
<td>Kynurenic acid</td>
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<td>4,6-Dihydroxyquinoline</td>
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Table 8. Proposed disturbances of urine metabolome and metabolic pathways (categorized by KEGG)

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<tr>
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<td>cis-Aconitic acid</td>
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<td></td>
<td>Isocitric acid</td>
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<tr>
<td></td>
<td>Citric acid</td>
<td></td>
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<tr>
<td>Nicotinate and nicotinamide metabolism</td>
<td>Niacinamide</td>
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<td>↓</td>
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<tr>
<td></td>
<td>Trigonelline</td>
<td>↓</td>
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<tr>
<td>Pyrimidine metabolism</td>
<td>Methylcytosine</td>
<td>↑</td>
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<tr>
<td></td>
<td>Urea</td>
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<tr>
<td></td>
<td>Thymidine</td>
<td>↑</td>
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<td></td>
<td>L-Glutamine</td>
<td></td>
<td>↑</td>
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<tr>
<td>Purine metabolism</td>
<td>Glyoxylic acid</td>
<td>↑</td>
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<tr>
<td></td>
<td>Adenosine 2',3'-cyclic phosphate</td>
<td>↑</td>
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<tr>
<td></td>
<td>L-Glutamine</td>
<td></td>
<td>↑</td>
<td></td>
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<tr>
<td>Aminobenzoate metabolism</td>
<td>3-Hydroxyanthranilnic acid</td>
<td></td>
<td>↑</td>
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<tr>
<td></td>
<td>Diethylphosphate</td>
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<tr>
<td></td>
<td>4-Aminophenol</td>
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<td></td>
<td>Mandelic acid</td>
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<tr>
<td></td>
<td>Aminobenzoic acid</td>
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</tbody>
</table>
Tryptophan metabolism is greatly disturbed in pre-diabetic state
**Fig. 10. Disturbances of tryptophan metabolism in HF group.**

↑ Red: increased  
↓ Blue: decreased
Fig. 11. Disturbances of tryptophan metabolism in HL group.

↑ Red: increased  
↓ Blue: decreased

Tryptophan → indoxyl → N-Formylkynurenine → kynurenine → 2-Aminobenzoic acid → Indoxyl

5-Hydroxytryptophan → Serotonin → 4,6-Dihydroxyquinoline → N-methyltryptamine → Indoleacetaldehyde

Kynurenate → Xanthurenic acid → N-Formylkynurenine → 3-Hydroxykynurenine → 3-Hydroxyanthranillic acid → 3-Hydroxyanthranillic acid → Quinolinate → NAD+

Tryptamine → 4,6-Dihydroxyquinoline → N-Acetylserotonin → Melatonin → 5-Hydroxyindoleacetic acid
Concentrations of tryptophan metabolites in urine

Quantitative analysis by UPLC triple quadrupole mass spectrometer was performed on urine samples. Data were presented as mean ± SD. *, P<0.05, **, P<0.01, ***, P<0.001 compared with CT group by one-way ANOVA with Tukey’s post-hoc test.

Is indoxyl sulfate a good biomarker for diabetic nephropathy (DN)?
Table 9. Disturbances in methylated metabolites

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>HF</th>
<th>HL</th>
<th>DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Methyltryptamine</td>
<td>↓</td>
<td>↓</td>
<td>—</td>
</tr>
<tr>
<td>5-Methoxyindoleacetate</td>
<td>↓</td>
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</tr>
<tr>
<td>Trigonelline</td>
<td>↓</td>
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<tr>
<td><strong>Methylhistidine</strong></td>
<td>↑</td>
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<tr>
<td>Methylimidazole acetaldehyde</td>
<td>↓</td>
<td>↓</td>
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<tr>
<td>Sarcosine</td>
<td>—</td>
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<td>↑</td>
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<tr>
<td><strong>Methylhippuric acid</strong></td>
<td>↑</td>
<td>↑</td>
<td>—</td>
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<tr>
<td>2-Methylbutyrylglycine</td>
<td>↓</td>
<td>↓</td>
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<tr>
<td><strong>Methylcytosine</strong></td>
<td>↑</td>
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<td>↓</td>
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<tr>
<td><strong>Dimethylguanosine</strong></td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td><strong>5-Methylcytidine</strong></td>
<td>↓</td>
<td>↓</td>
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<tr>
<td>5-Methyldeoxyctydine</td>
<td>—</td>
<td>—</td>
<td>↓</td>
</tr>
<tr>
<td>2(N)-Methyl-norsalsolinol</td>
<td>↓</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4-Methylcatechol</td>
<td>↓</td>
<td>↓</td>
<td>—</td>
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<tr>
<td><strong>1-Methylhypoxanthine</strong></td>
<td>—</td>
<td>↑</td>
<td>—</td>
</tr>
<tr>
<td><strong>Methyluric acid</strong></td>
<td>—</td>
<td>—</td>
<td>↓</td>
</tr>
<tr>
<td><strong>Methylguanin</strong></td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Methylglutaconic acid</td>
<td>—</td>
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<td>↑</td>
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</tbody>
</table>
From pre-diabetic state to T2DM and diabetes complications

Muscle: Protein wasting

- Fatty streaks
- Atherosclerosis

Metabolic syndrome

- Obesity (central obesity)
- IR and MS

Liver:

- Non-alcoholic fatty liver disease (NAFLD)
- NASH
- Liver fibrosis

Muscle:

- Non-alcoholic fatty liver disease (NAFLD)
- NASH
- Liver fibrosis

- Fatty streaks
- Atherosclerosis and CVD


c. Liver fibrosis

Kanter et al. Circ Res. 2007;100:769-781

Hyperglycemia
Dyslipidemia
Inflammation
Oxidative stress
A metabolomics-based community cohort study to search for biomarkers of pre-diabetic state (metabolic syndrome)

RESEARCH DESIGN AND METHODS:

Urine metabolomics has potential utility in metabolic profiling because urine metabolites analysis reflects global outflux of metabolic change. We collected data on subjects (n = 99) with overweight, dyslipidemia, hypertension or impaired glucose tolerance and took a metabolomics approach to analyze the metabolites of urine by HPLC–TOF MS and elicit potential biomarkers to picture metabolic syndrome.

A community cohort study as proof of concept
RESULTS:

a. Results revealed that the urine **nicotinuric acid** value of subjects with diabetes was higher than subjects without diabetes.

b. Moreover, urinary **nicotinuric acid** level was positively correlated with BMI, blood pressure, total cholesterol, LDL-C, triacylglycerol and high sensitivity CRP, but negatively correlated with HDL-C.

CONCLUSION:
This is the first study to propose that nicotinuric acid represents an important pathogenic mechanism in process from metabolic syndrome to diabetes and atherosclerotic cardiovascular disease.

What is nicotinuric acid?

a. Nicotinuric acid is a **glycine** conjugate of **nicotinic acid**.

b. Nicotinic acid is a catabolic metabolite of **NAD⁺**, which is derived from **tryptophan**.

Nicotinic acid + Glycine
Tryptophan metabolism

Tryptophan → indoxyl → N-Formylkynurenine → kynurenine → Kynurenate

Tryptophan → 5-Hydroxytryptophan → N-Methyltryptamine → Indoleacetaldehyde

Tryptophan → 3-Hydroxykynurenine → 2-Aminobenzoic acid

Tryptophan → 3-Hydroxyanthranilic acid → 6-Hydroxymelatonin

Tryptophan → 4,6-Dihydroxyquinoline

Tryptophan → Formylkynurenine → Formylanthranilic acid

Tryptophan → 3-Hydroxyanthranilic acid

Tryptophan → NAD+ → Nicotinuric acid

Serotonin → N-Acetylserotonin

Serotonin → N-Methylserotonin

Serotonin → Melatonin → 5-Hydroxyindoleacetic acid

Serotonin → Kynurenate → Xanthurenic acid

Serotonin → Quinolinate

Serotonin → 5-Hydroxytryptophan
Portfolio of biomarkers for metabolic syndrome (a pre-diabetic state) (proposed)

a. Tryptophan metabolism (catabolic metabolites): nicotinuric acid
b. Branched-chain amino acids (BCAAs): valine, leucine, and isoleucine
c. Acylcarnitines: C$_3$-C$_5$ acylcarnitines
d. Disturbed methylation-demethylation

Chen A., Cheng ML, and Shiao MS, unpublished results
Healthy Ageing: What are we looking for?

>>An hypothesis-generating approach to search for healthy ageing-associated metabolites and early sign of degenerative diseases (diabetes)

A healthy, aged community
A similar dietary habit and lifestyle
Good record of medical care

Chang Gung Health & Culture Village (CG-HACV)
Urine metabolome: analyzed by 600 MHz $^1$H-NMR

Fig. 1. Principal component analysis (PCA) (score plot)

Metabolites are highly clustered
Fig. 1. Targeted metabolites (based on PCA Score plot) identification by LC-MS and database

D-Glucose (5.2, 4.6, 3.9, 3.7, 3.5, 3.4, 3.2)
Sucrose (5.4, 4.2, 4.0, 3.9, 3.7, 3.6, 3.5)
Salicylate (8.9, 7.8, 7.5, 7.0, 7.0, 4.0)
Hippurate (8.5, 7.8, 7.6, 7.5, 4.0)
Summary of findings: not just ours

a. Keep away from pre-diabetes (metabolic syndrome) and T2DM
b. Have good **gut microflora**
c. Maintain functional **energy metabolism**
1. Are there phytochemicals or food factors to promote healthy ageing by preventing degenerative diseases such as diabetes?
   a. Resveratrol
   b. Bitter gourd
   c. EGCG (in Tea)

2. How to build a healthy gut microflora?

>>From 85 yr (life span) to 122 yr (life expectancy), what can be done?
Metabolomics Core Laboratory, Healthy Ageing Research Center, Chang Gung University

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Thanks for your kind attention

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National Science Council, Taiwan