UPLC-Q-TOF/MS-based metabonomic studies on the intervention effects of aspirin eugenol ester in atherosclerosis hamsters

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**Table S1** Optimized gradient elution program of UPLC-Q-TOF/MS in plasma and urine metabonomic analysis.

| Time (min) | Plasma |              | Time (min) | Urine |              |
|------------|--------|--------------|------------|-------|--------------|
|            | Solvent A (%) | Solvent B (%) |            | Solvent A (%) | Solvent B (%) |
| 0          | 95     | 5            | 0          | 98    | 2            |
| 3          | 60     | 40           | 0.5        | 98    | 2            |
| 7          | 40     | 60           | 7          | 80    | 20           |
| 9          | 15     | 85           | 9          | 65    | 35           |
| 16         | 5      | 95           | 13         | 30    | 70           |
| 20         | 5      | 95           | 15         | 2     | 98           |
|            |        |              |            | 17    | 2            |
|            |        |              |            | 19    | 98           |
|            |        |              |            | 20    | 98           |

Solvent A: water with 0.1% formic acid (by volume); Solvent B: acetonitrile with 0.1% formic acid (by volume).
Table S2 Pathway analysis result with MetaboAnalyst 3.0 of the potential biomarkers in plasma and urine.

| No. | Pathway name                                      | Total | Expected  | Hits | Raw p   | -log(P) | Impact   |
|-----|--------------------------------------------------|-------|-----------|------|---------|---------|----------|
| 1   | Valine, leucine and isoleucine biosynthesis      | 11    | 0.13338   | 2    | 0.007141| 4.9419  | 0.42857  |
| 2   | Glyoxylate and dicarboxylate metabolism          | 16    | 0.19401   | 1    | 0.1782  | 1.7249  | 0.16667  |
| 3   | Pantothenate and CoA biosynthesis                | 15    | 0.18188   | 2    | 0.013249| 4.3238  | 0.15384  |
| 4   | Riboflavin metabolism                            | 11    | 0.13338   | 1    | 0.126   | 2.0714  | 0.15384  |
| 5   | Lysine degradation                               | 20    | 0.24251   | 1    | 0.21782 | 1.5241  | 0.0625   |
| 6   | Nicotinate and nicotinamide metabolism           | 13    | 0.15763   | 1    | 0.14724 | 1.9157  | 0.05556  |
| 7   | Glycerophospholipid metabolism                   | 30    | 0.36377   | 1    | 0.30916 | 1.1739  | 0.05263  |
| 8   | Aminoacyl-tRNA biosynthesis                      | 67    | 0.81241   | 2    | 0.19338 | 1.6431  | 0.04166  |
| 9   | Valine, leucine and isoleucine degradation       | 38    | 0.46077   | 2    | 0.075344| 2.5857  | 0.03922  |
| 10  | Citrate cycle (TCA cycle)                        | 20    | 0.24251   | 1    | 0.21782 | 1.5241  | 0.03448  |
| 11  | Glutathione metabolism                           | 26    | 0.31526   | 1    | 0.2739  | 1.295   | 0.02941  |
| 12  | Arginine and proline metabolism                  | 44    | 0.53352   | 1    | 0.4203  | 0.86679 | 0.02041  |
| 13  | Biosynthesis of unsaturated fatty acids          | 42    | 0.50927   | 2    | 0.08971 | 2.4112  | 0        |
| 14  | beta-Alanine metabolism                          | 19    | 0.23072   | 1    | 0.20809 | 1.5698  | 0        |
| 15  | Fatty acid biosynthesis                          | 43    | 0.5214    | 1    | 0.41296 | 0.88441 | 0        |

Total: The total number of compounds in the pathways; the hits are the actually matched number from the user upload data; the raw p is the original p value calculated from the enrichment analysis; the impact is the pathway impact value calculated from pathway analysis.
Figure S1 Representative TICs of the plasma samples analyzed by UPLC-Q-TOF in positive and negative modes.

Retention time and abundance percentage were labeled upon the peaks.
Fig. S2 Clustering heatmap of potential biomarkers in plasma associated with atherosclerosis and AEE treatment.

Differences of samples and metabolites were hierarchically clustered. Increasing expression values were coded with green to red colors. Rows indicated potential biomarkers; columns indicated samples.
Figure S3 Representative TICs of the urine samples analyzed by UPLC-Q-TOF in positive and negative modes.

Retention time and abundance percentage were labeled upon the peaks.