Supplementary Material

Supplementary Methods

Sample Collection and Platelet Function Testing in HAPI

At the initial clinic visit, baseline whole blood platelet aggregometric studies were performed in the fasting state. The aspirin intervention began the day after this visit and continued for 14 days. One to 3 days before the second clinic visit a home visit was conducted to ensure compliance. Compliance was again assessed at the second clinic visit by pill count and review of the subjects study diary.

Venous blood (9 ml) for platelet aggregation studies was collected from the antecubital vein by gentle aspiration using a 21-gauge butterfly cannula and a 10-ml sterile syringe charged with 0.105 M sodium citrate anticoagulant 1 ml. Whole blood platelet impedance aggregometry was performed within 3 hours after blood was drawn.

Whole blood platelet impedance aggregometry was done using a Chrono-Log 4-channel aggregometer (Chrono-Log,Havertown, Pennsylvania) within 3 hours after blood was drawn. Instrument incubation wells were set to 37°C, and stirring speed set at 1,000 rpm. Prewarmed cuvettes were filled with Hank's Balanced Salt Solution 0.5 ml (Sigma-Aldrich, St. Louis, Missouri), citrate anticoagulated whole blood 0.5 ml, and a stir bar. After a 5-minute incubation period, a prewarmed probe was inserted into each cuvette and the cuvettes were moved to reaction wells. The aggregation baseline was set to 0 and the impedance circuit was calibrated to 50%. Aggregation was initiated with the addition of agonists, for the current analysis collagen or arachidonic acid, which was purchased from Chrono-Log (Horsham, Pennsylvania). Each channel of the 4-channel aggregometer was dedicated to a particular agonist. Reactions were allowed to run for 10 minutes, but all calculations were based on a 5-minute test time.

On the 14th day, the participant took aspirin approximately 1 hour before arriving at the clinic, when fasting blood was drawn, and whole blood platelet aggregometric studies were repeated in identical fashion to baseline and by the same technician.

<u>Statistics: Linear Models and Linear Mixed Models</u>

We assessed the significance of the effects of aspirin exposure and sex on metabolite level using linear models and linear mixed models in GenStat 14th edition (VSN International, Hemel Hempstead, UK). Three models were fitted for each metabolite.

The first global linear mixed model included all data for each metabolite:

(1): $y_{i,t} = \alpha_0 + \alpha_1 * t + \alpha_2 * sex_i + + \alpha_3 * t * sex_i + \delta_i + \varepsilon_{i,t}$

in which i=1,2…156, $y_{i,t}$=vector of metabolite concentrations for the ith individual at time t; t=time (pre- or post-aspirin exposure), sex=man or woman, fixed effects; $\alpha_0…\alpha_3$=regression coefficients; δ_i= random effect associated with the ith individual; $\varepsilon_{i,t}$=error term.

The second and third linear models included data before and after treatment resp.:

(2): $y = \beta_0 + \beta_1 \, sex$

(3:) $y = \gamma_0 + \gamma_1 \, sex$

The effect of the aspirin intervention on metabolite level was considered significant when $p < 0.05$ for α_1. The effect of sex was considered significant before, after and during treatment when $p < 0.05$ for β_1, γ_1 or α_3 respectively.

Figure S1: Oxylipid biosynthetic pathways (adapted from Strassburg et al[1])

Metabolites detected in our samples are in bold.
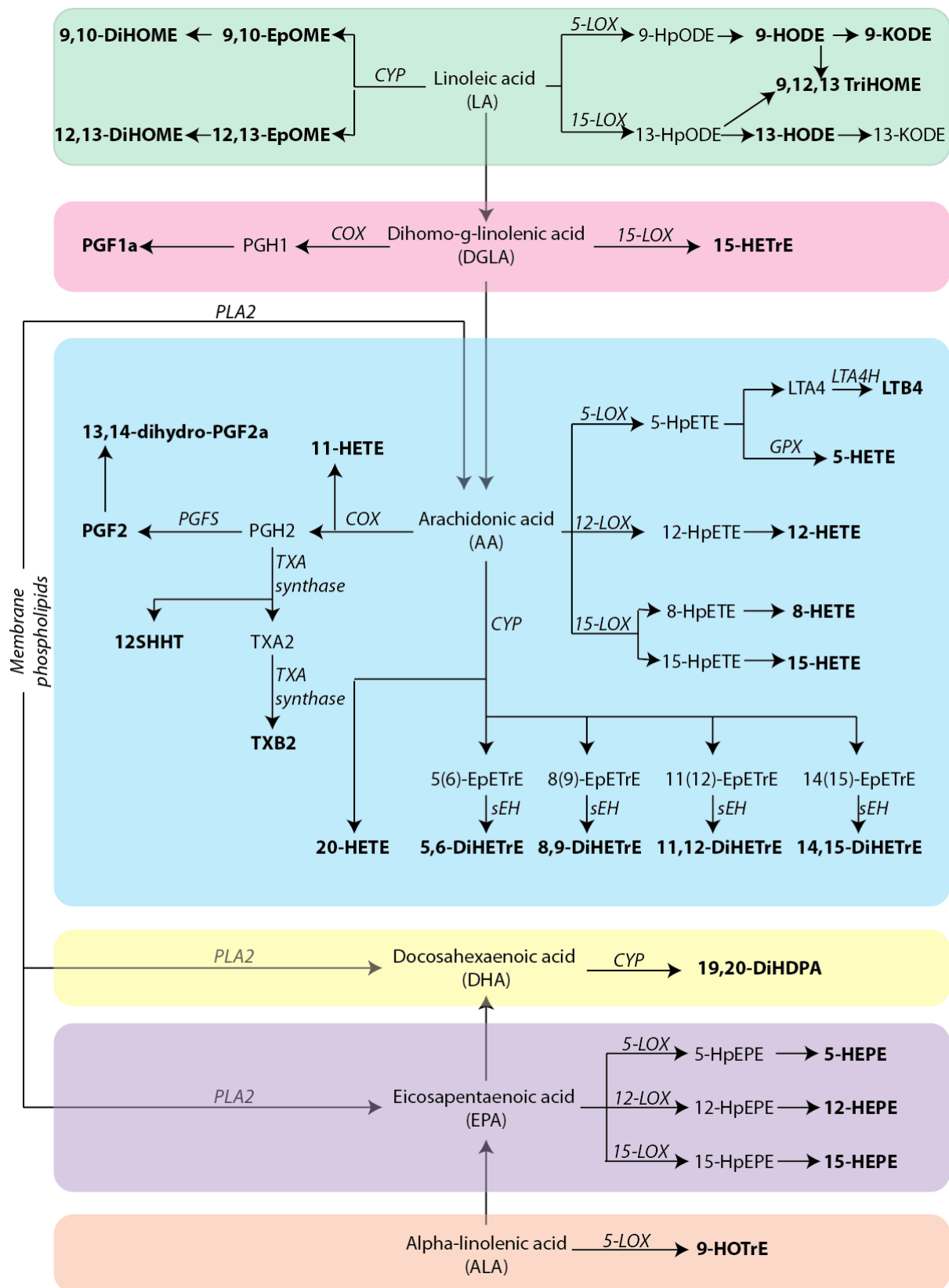
Supplementary reference

1. Strassburg, K., A. M. L. Huijbrechts, K. A. Kortekaas, J. H. Lindeman, T. L. Pedersen, A. Dane, R. Berger, A. Brenkman, T. Hankemeier, J. van Duynhoven, E. Kalkhoven, J. W. Newman, and R. J. Vreeken. Quantitative profiling of oxylipins through comprehensive LC-MS/MS analysis: application in cardiac surgery. *Anal Bioanal Chem*. 2012;404:1413–1426.