Electronic Supplementary Material (ESI) for Analyst.


# Electronic Supplementary Information

## Clinical blood sampling for oxylipin analysis
–
Effect of storage and pneumatic tube transport of blood on free and total oxylipin profile in human plasma and serum

Katharina M. Rund, Fabian Nolte, Julian Doricic, Robert Greite, Sebastian Schott, Ralf Lichtinghagen, Faikah Gueler, Nils Helge Schebb

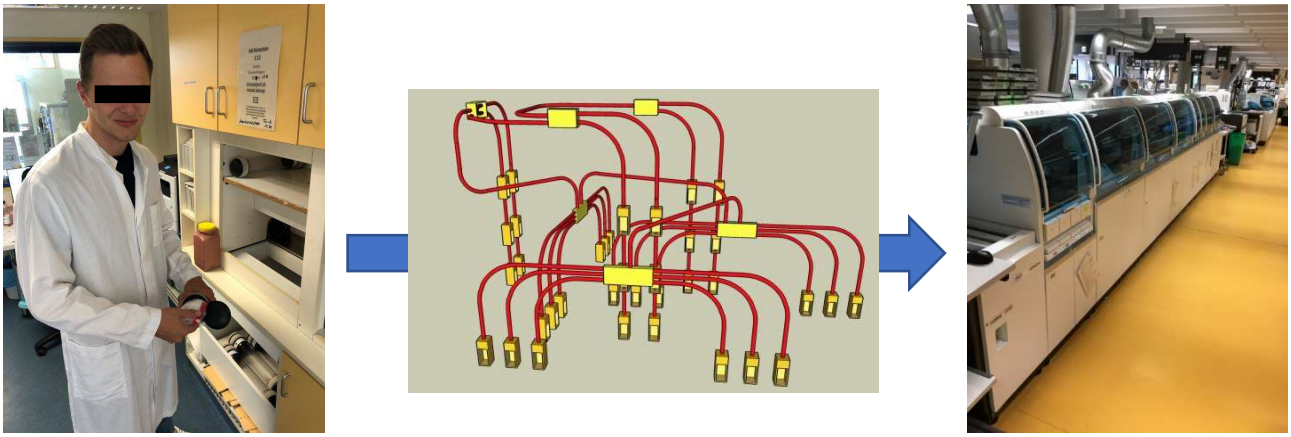



**Fig. S1:** Overview of the pneumatic tube system transport at the Hannover Medical School. Samples collected on the ward or the intensive care unit are packed in tubes and transferred via the pneumatic tube system transport which covers in total about 50 km pipe distance to the clinical chemistry laboratory where continuous (24/7) sample processing is carried out.

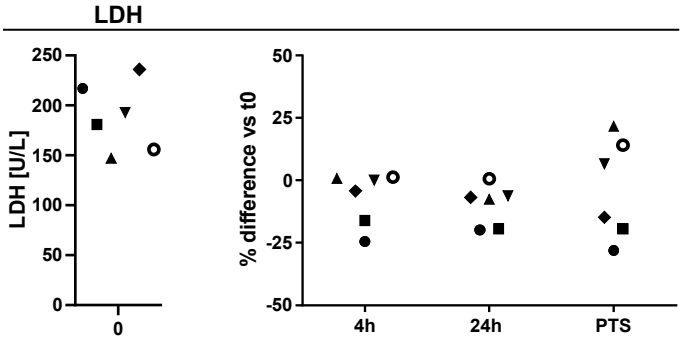



**Fig. S2:** Lactate dehydrogenase (LDH) in plasma. Shown are individual levels (n=6) of LDH at *t0* (immediate processing) and % difference *vs. t0* after storage for 4 h at 4 °C and 24 h at 4 °C or after pneumatic tube system transport before centrifugation to generate plasma. The different symbols represent samples from different individual human subjects.
LDH was analyzed by an Olympus analyzer (AU400) in an automated fashion according to the manufacturer's instruction.

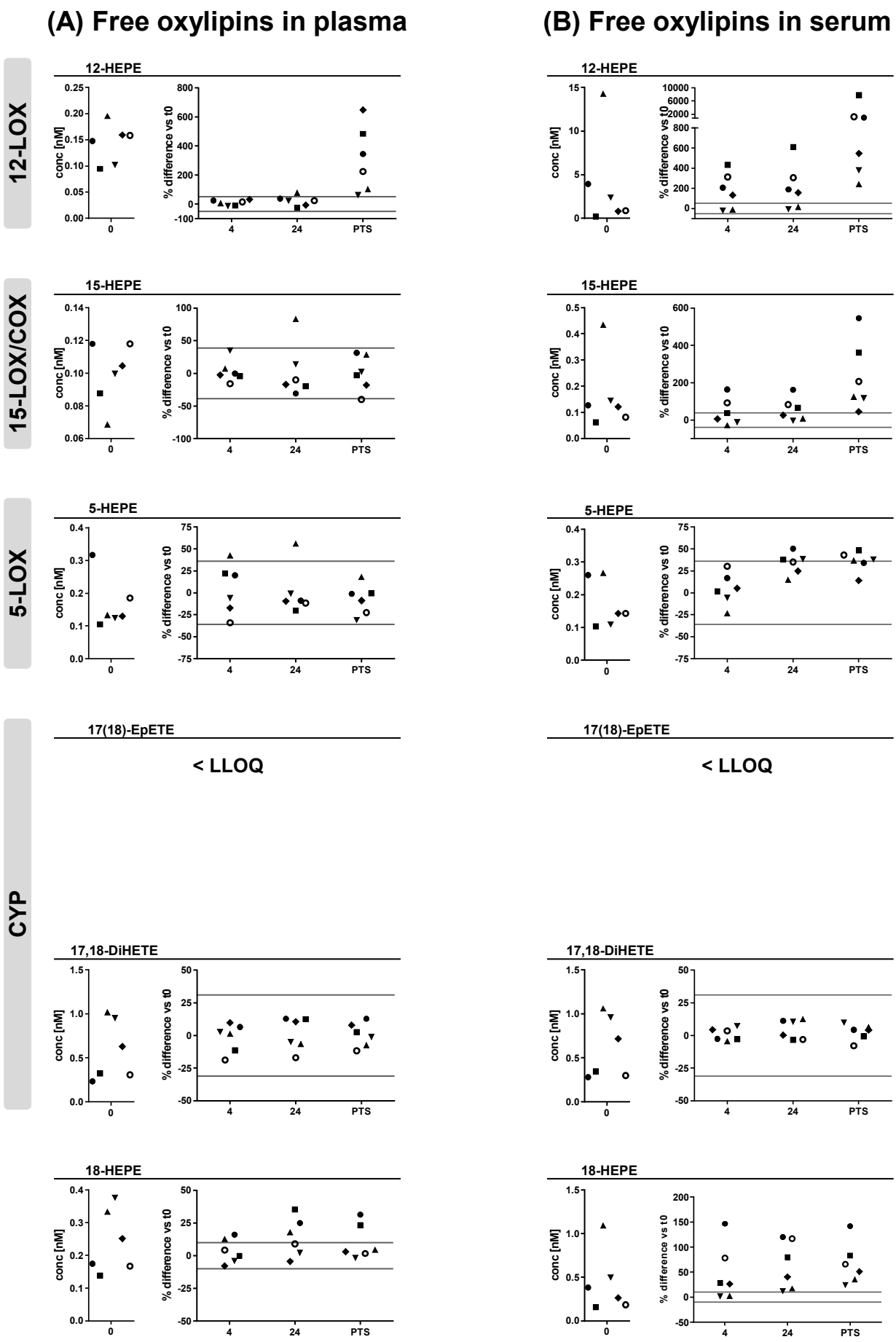



**Fig. S3:** EPA derived free oxylipins in **(A)** plasma and **(B)** serum. Shown are individual concentrations (n=6) of selected oxylipins from major formation pathways at *t0* (immediate processing) and % difference *vs. t0* after storage for 4 h at 4 °C and 24 h at 4 °C or after pneumatic tube system transport before centrifugation to generate plasma or serum. The different symbols represent samples from different individual human subjects. The grey lines indicate acceptable change limits calculated based on relative SD of quality control plasma (summarized in Tab.1).

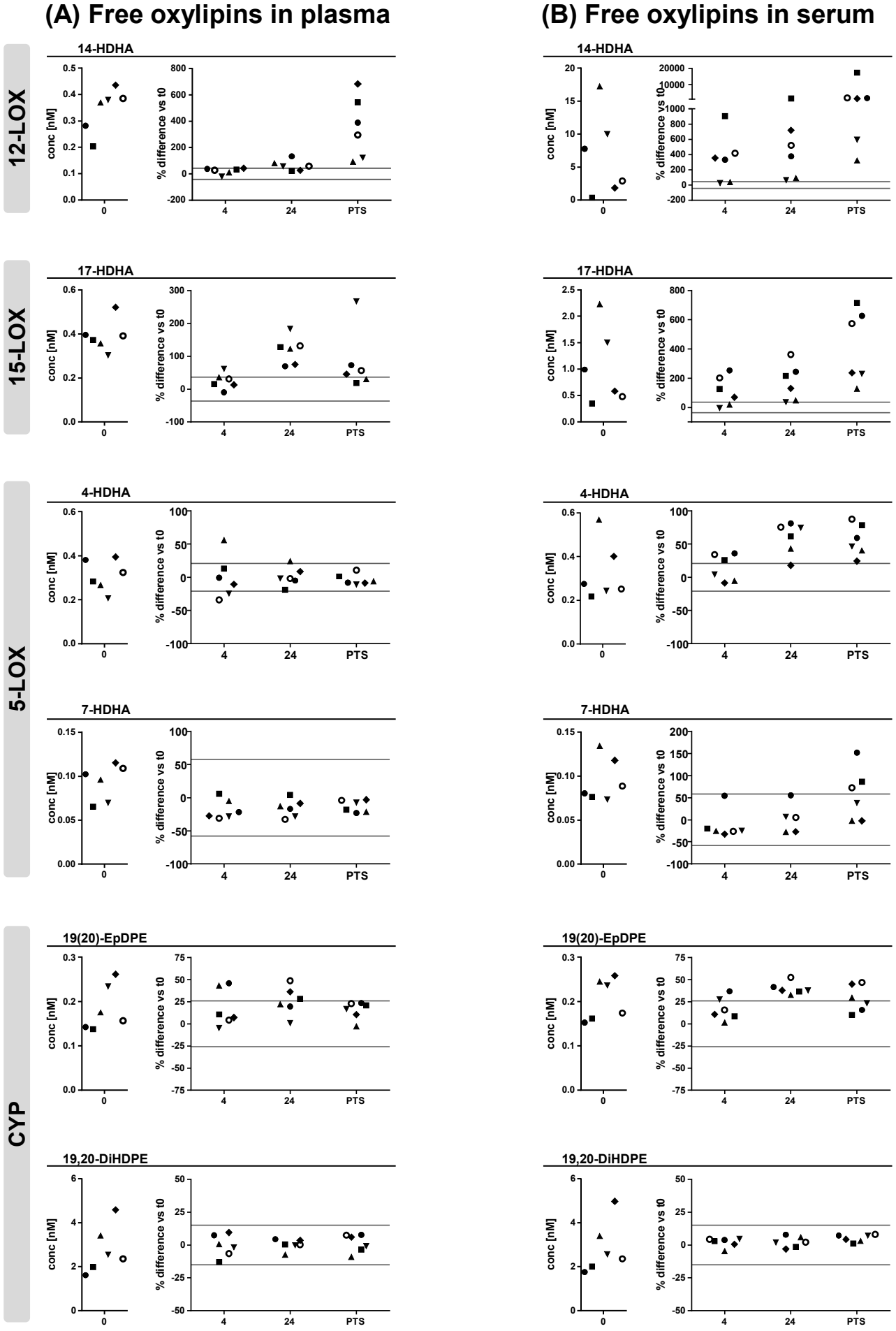



**Fig. S4:** DHA derived free oxylipins in **(A)** plasma and **(B)** serum. Shown are individual concentrations (n=6) of selected oxylipins from major formation pathways at *t0* (immediate processing) and % difference *vs. t0* after storage for 4 h at 4 °C and 24 h at 4 °C or after pneumatic tube system transport before centrifugation to generate plasma or serum. The different symbols represent samples from different individual human subjects. The grey lines indicate acceptable change limits calculated based on relative SD of quality control plasma (summarized in Tab.1).

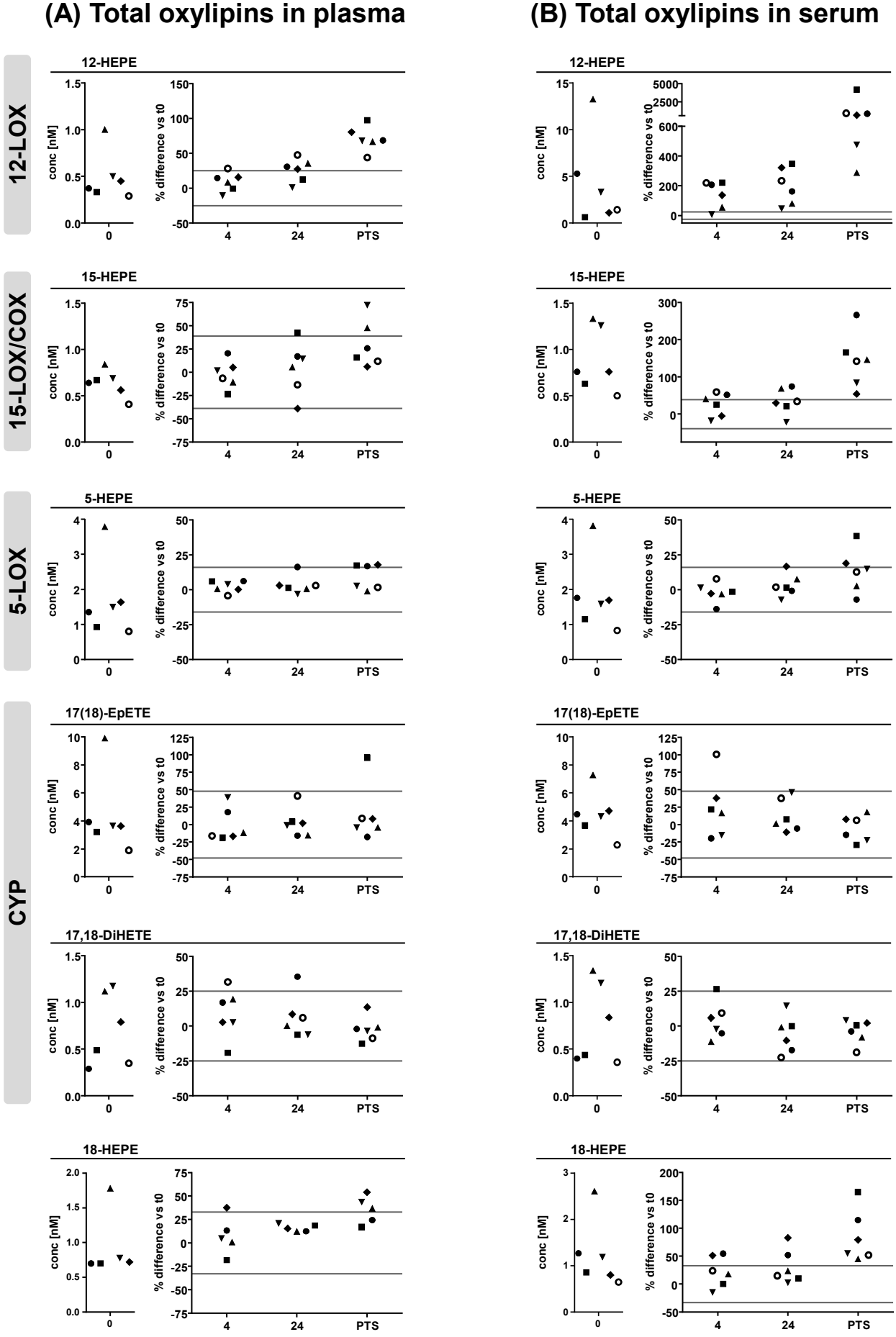



**Fig. S5:** EPA derived total (i.e. sum of free and esterified) oxylipins in **(A)** plasma and **(B)** serum. Shown are individual concentrations (n=6) of selected oxylipins from major formation pathways at *t0* (immediate processing) and % difference *vs. t0* after storage for 4 h at 4 °C and 24 h at 4 °C or after pneumatic tube system transport before centrifugation to generate plasma or serum. The different symbols represent samples from different individual human subjects. The grey lines indicate acceptable change limits calculated based on relative SD of quality control plasma (summarized in Tab.1).

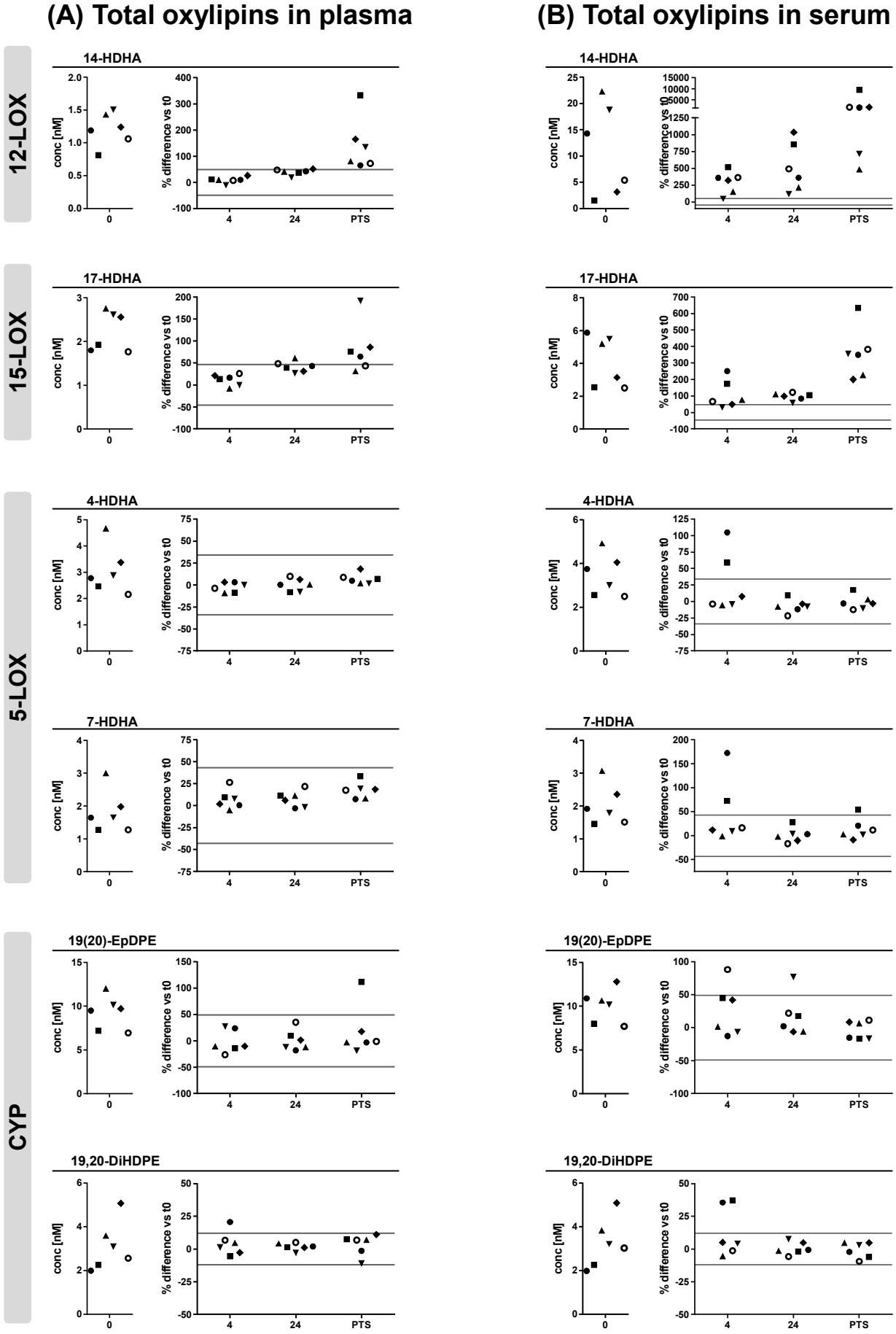
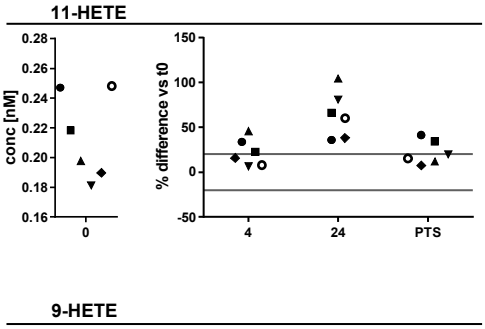



**Fig. S6:** DHA derived total oxylipins in **(A)** plasma and **(B)** serum. Shown are individual concentrations (n=6) of selected oxylipins from major formation pathways at *t0* (immediate processing) and % difference *vs. t0* after storage for 4 h at 4 °C and 24 h at 4 °C or after pneumatic tube system transport before centrifugation to generate plasma or serum. The different symbols represent samples from different individual human subjects. The grey lines indicate acceptable change limits calculated based on relative SD of quality control plasma (summarized in Tab.1).

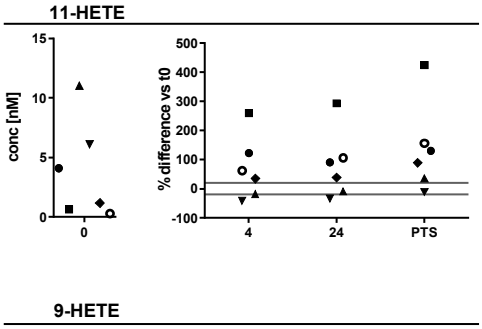
## (A) Free oxylipins in plasma




9-HETE

< LLOQ

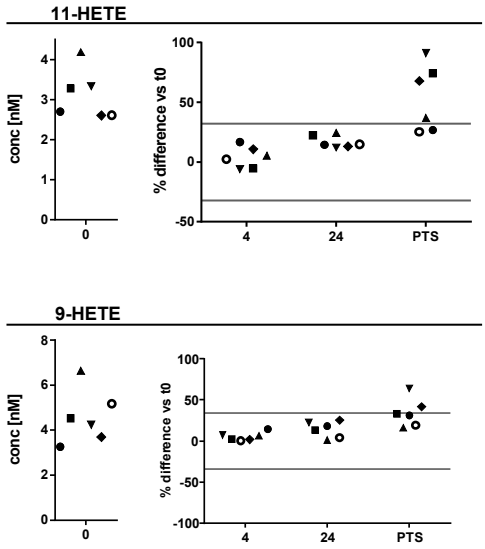
## (B) Free oxylipins in serum




9-HETE

< LLOQ

## (C) Total oxylipins in plasma




## (D) Total oxylipins in serum

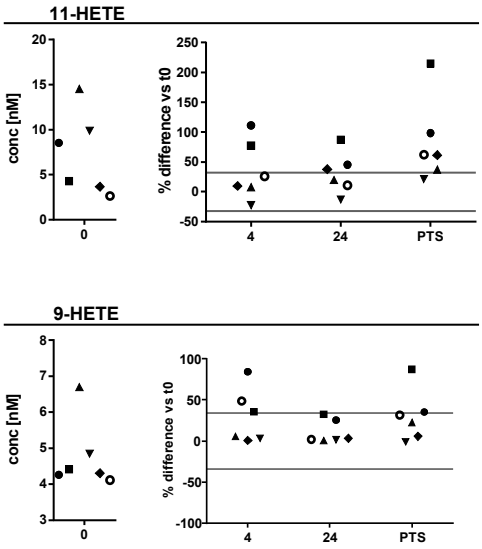



**Fig. S7:** ARA derived 11-HETE and 9-HETE in **(A+C)** plasma and **(B+D)** serum. Shown are individual concentrations (n=6) of **(A+B)** free or **(C+D)** total (comprising free and esterified) mediators at *t0* (immediate processing) and % difference *vs. t0* after storage for 4 h at 4 °C and 24 h at 4 °C or after pneumatic tube system transport before centrifugation to generate plasma or serum. The different symbols represent samples from different individual human subjects. The grey lines indicate acceptable change limits calculated based on relative SD of quality control plasma (summarized in Tab.1).

Tab. S1: Median concentrations with interquartil range (25% percentil, 75% percentil) [nM] of representative free oxylipins in plasma and serum following direct processing, after storage for 4 h or 24 h at 4 °C, or after pneumatic tube system transport (PTS) prior to centrifgation (n =6).

| | plasma median (25% percentil, 75% percentil) [nM] | | | | serum median (25% percentil, 75% percentil) [nM] | | | |
|---|---|---|---|---|---|---|---|---|
| | direct | 4 h | 24 h | PTS | direct | 4 h | 24 h | PTS |
| $PGE_2$ | 0.010 (0.010, 0.039) | 0.015 (0.014, 0.036) | 0.022 (0.017, 0.038) | 0.040 (0.033, 0.068) | 1.3 (0.27, 1.8) | 0.98 (0.50, 2.0) | 0.98 (0.43, 1.6) | 1.2 (0.78, 2.4) |
| $TxB_2$ | 0.13 (0.078, 0.24) | 0.21 (0.11, 0.32) | 0.29 (0.18, 0.39) | 0.56 (0.39, 0.78) | 53 (9.1, 142) | 69 (21, 168) | 62 (21, 145) | 73 (32, 167) |
| 12-HHT | 0.18 (0.15, 0.29) | 0.24 (0.18, 0.35) | 0.40 (0.35, 0.60) | 0.65 (0.46, 0.79) | 50 (8.6, 104) | 53 (21, 133) | 59 (20, 142) | 67 (29, 158) |
| 12-HETE | 2.3 (1.1, 3.5) | 2.5 (2.2, 4.7) | 5.4 (2.1, 7.0) | 5.8 (5.6, 7.8) | 97 (22, 209) | 114 (67, 273) | 116 (74, 259) | 312 (224, 538) |
| 12-HEPE | 0.15 (0.10, 0.17) | 0.18 (0.087, 0.20) | 0.17 (0.11, 0.24) | 0.53 (0.34, 0.79) | 1.6 (0.66, 6.5) | 2.8 (1.7, 12) | 2.9 (2.1, 13) | 14 (10, 44) |
| 14-HDHA | 0.37 (0.26, 0.40) | 0.40 (0.30, 0.52) | 0.61 (0.48, 0.66) | 1.3 (0.82, 2.0) | 5.3 (1.5, 12) | 14 (7.2, 26) | 17 (13, 34) | 68 (47, 88) |
| 15-HETE | 0.71 (0.66, 0.90) | 0.80 (0.77, 1.0) | 0.97 (0.77, 1.1) | 0.88 (0.73, 1.2) | 3.7 (1.2, 10) | 4.5 (2.8, 13) | 5.5 (2.9, 14) | 9.1 (4.4, 19) |
| 15-HEPE | 0.10 (0.083, 0.12) | 0.10 (0.081, 0.12) | 0.097 (0.079, 0.12) | 0.087 (0.082, 0.012) | 0.12 (0.077, 0.22) | 0.14 (0.12, 0.32) | 0.17 (0.15, 0.37) | 0.30 (0.23, 0.86) |
| 17-HDHA | 0.38 (0.34, 0.43) | 0.49 (0.41, 0.53) | 0.85 (0.76, 0.91) | 0.65 (0.46, 0.85) | 0.79 (0.45, 1.7) | 1.4 (0.94, 2.9) | 2.1 (1.2, 3.3) | 4.1 (2.6, 5.6) |
| 5-HETE | 0.58 (0.46, 0.91) | 0.65 (0.52, 1.86) | 0.72 (0.63, 0.92) | 0.57 (0.53, 0.89) | 1.4 (1.1, 2.0) | 1.5 (1.2, 2.0) | 2.0 (1.5, 2.5) | 2.1 (1.8, 3.7) |
| 5-HEPE | 0.13 (0.12, 0.22) | 0.13 (0.12, 0.24) | 0.14 (0.11, 0.23) | 0.13 (0.10, 0.20) | 0.14 (0.11, 0.26) | 0.17 (0.11, 0.23) | 0.19 (0.15, 0.33) | 0.18 (0.15, 0.35) |
| 4-HDHA | 0.30 (0.25, 0.38) | 0.34 (0.20, 0.39) | 0.33 (0.22, 0.38) | 0.32 (0.23, 0.36) | 0.26 (0.24, 0.44) | 0.35 (0.27, 0.42) | 0.46 (0.36, 0.58) | 0.46 (0.38, 0.57) |
| 7-HDHA | 0.099 (0.069, 0.11) | 0.078 (0.064, 0.086) | 0.078 (0.064, 0.090) | 0.077 (0.062, 0.11) | 0.084 (0.076, 0.12) | 0.073 (0.060, 0.11) | 0.090 (0.071, 0.10) | 0.14 ( 0.11, 0.17) |
| 14(15)-EpETrE | 0.058 (0.048, 0.067) | 0.063 (0.055, 0.070) | 0.072 (0.045, 0.079) | 0.059 (0.047, 0.065) | 0.079 (0.071, 0.097) | 0.090 (0.071, 0.11) | 0.087 (0.072, 0.15) | 0.090 (0.080, 0.093) |
| 17(18)-EpETE | < LLOQ | | | | < LLOQ | | | |
| 19(20)-EpDPE | 0.17 (0.14, 0.24) | 0.22 (0.16, 0.26) | 0.22 (0.18, 0.27) | 0.18 (0.17, 0.28) | 0.21 (0.16, 0.25) | 0.23 (0.20, 0.29) | 0.30 (0.20, 0.33) | 0.27 (0.18, 0.33) |
| 14,15-DiHETrE | 0.59 (0.54, 0.64) | 0.60 (0.51, 0.65) | 0.58 ( 0.54, 0.67) | 0.63 (0.54, 0.68) | 0.60 (0.56, 0.71) | 0.61 (0.57, 0.73) | 0.62 (0.56, 0.72) | 0.64 (0.59, 0.72) |
| 17,18-DiHETE | 0.48 (0.29, 0.97) | 0.49 (0.25, 0.99) | 0.53 (0.26, 0.92) | 0.51 (0.27, 0.94) | 0.53 (0.29, 0.99) | 0.54 (0.30, 1.0) | 0.59 (0.31, 1.1) | 0.55 (0.29, 1.1) |
| 19,20-DiHDPE | 2.5 (1.9, 3.7) | 2.4 (1.7, 3.8) | 2.5 (1.9, 3.6) | 2.5 ( 1.9, 3.5) | 2.5 (1.9, 3.8) | 2.6 (2.0, 3.7) | 2.5 (1.9, 3.9) | 2.7 (2.0, 3.9) |
| 5(*R,S*)-5-$F_{2t}$-IsoP | 0.092 (0.072, 0.11) | 0.086 (0.064, 0.11) | 0.097 (0.085, 0.13) | 0.088 (0.071, 0.097) | 0.094 (0.082, 0.11) | 0.099 (0.086, 0.12) | 0.11 (0.10, 0.16) | 0.11 (0.096, 0.13) |
| 9-HETE | < LLOQ | | | | < LLOQ | | | |
| 11-HETE | 0.21 (0.19, 0.25) | 0.27 (0.21, 0.30) | 0.35 (0.31, 0.40) | 0.25 (0.21, 0.31) | 2.6 (0.56, 7.4) | 3.0 (1.3, 9.1) | 3.5 (1.4, 8.4) | 4.4 (1.9, 11) |
| 18-HEPE | 0.21 (0.16, 0.34) | 0.22 (0.17, 0.37) | 0.23 (0.19, 0.39) | 0.24 (0.17, 0.35) | 0.32 (0.18, 0.65) | 0.42 (0.29, 0.98) | 0.48 (0.33, 0.95) | 0.51 (0.30, 1.1) |

Tab. S2: Median concentrations with interquartil range (25% percentil, 75% percentil) [nM] of representative total, i.e. sum of free and esterified oxylipins in plasma and serum following direct processing, after storage for 4 h or 24 h at 4 °C, or after pneumatic tube system transport (PTS) prior to centrifugation (n =6).

| | plasma median (25% percentil, 75% percentil) [nM] | | | | serum median (25% percentil, 75% percentil) [nM] | | | |
|---|---|---|---|---|---|---|---|---|
| | direct | 4 h | 24 h | PTS | direct | 4 h | 24 h | PTS |
| $PGE_2$ * | - | - | - | - | - | - | - | - |
| $TxB_2$ * | - | - | - | - | - | - | - | - |
| 12-HHT | 0.43 (0.37, 0.55) | 0.51 (0.39, 0.64) | 0.66 (0.56, 0.77) | 1.1 (0.71, 1.2) | 82 (14, 187) | 87 (28, 243) | 85 (31, 209) | 105 (44, 234) |
| 12-HETE | 5.4 (4.1, 7.9) | 5.9 (5.2, 9.5) | 9.0 (6.1, 12) | 12 (11, 13) | 134 (25, 255) | 159 (88, 451) | 176 (100, 396) | 644 (336, 892) |
| 12-HEPE | 0.41 (0.32, 0.63) | 0.44 (0.36, 0.66) | 0.50 (0.41, 0.77) | 0.73 (0.57, 1.1) | 2.4 (0.97, 7.3) | 4.1 (2.4, 17) | 4.8 (4.1, 16) | 22 (13, 52) |
| 14-HDHA | 1.2 (1.0, 1.5) | 1.3 (1.1, 1.6) | 1.8 (1.5, 1.9) | 2.9 (1.9, 3.5) | 9.8 (2.7, 20) | 26 (12, 58) | 39 (27, 66) | 138 (84, 174) |
| 15-HETE | 6.2 (5.6, 7.1) | 6.1 (5.6, 7.4) | 6.9 (6.1, 7.9) | 8.9 (7.1, 11) | 11 (7.1, 19) | 15 (9.3, 27) | 17 (10, 26) | 39 (19, 44) |
| 15-HEPE | 0.65 (0.52, 0.73) | 0.64 (0.48, 0.76) | 0.77 (0.35, 0.90) | 0.79 (0.56, 1.2) | 0.76 (0.60, 1.3) | 0.92 (0.78, 1.3) | 0.99 (0.74, 1.6) | 2.0 (1.2, 2.9) |
| 17-HDHA | 2.2 (1.8, 2.7) | 2.4 (2.2, 2.7) | 3.0 (2.6, 3.6) | 3.5 (2.9, 5.5) | 4.2 (2.5, 5.6) | 7.1 (4.6, 12) | 7.5 (5.5, 11) | 18 (11, 25) |
| 5-HETE | 14 (13, 16) | 13 (13, 16) | 13 (13, 16) | 15 (13, 18) | 16 (15, 18) | 19 (16, 21) | 15 (14, 20) | 17 (16, 22) |
| 5-HEPE | 1.4 (0.90, 2.2) | 1.5 (0.93, 2.2) | 1.5 (0.92, 2.2) | 1.6 (1.0, 2.4) | 1.6 (1.1, 2.3) | 1.6 (1.1, 2.2) | 1.6 (1.1, 2.5) | 1.7 (1.4, 2.5) |
| 4-HDHA | 2.8 (2.4, 3.7) | 2.9 (2.2, 3.7) | 2.7 (2.3, 3.9) | 2.9 (2.6, 4.2) | 3.4 (2.5, 4.3) | 4.2 (2.8, 5.4) | 3.1 (2.6, 4.1) | 3.3 (2.6, 4.2) |
| 7-HDHA | 1.7 (1.3, 2.2) | 1.7 (1.6, 2.2) | 1.6 (1.5, 2.4) | 1.9 (1.7, 2.6) | 1.9 (1.5, 2.5) | 2.6 (1.9, 3.6) | 1.9 (1.7, 2.3) | 2.2 (1.8, 2.5) |
| 14(15)-EpETrE | 45 (38, 58) | 51 (27, 55) | 44 (37, 53) | 40 (37, 68) | 44 (41, 64) | 53 ( 44, 76) | 59 (49, 68) | 46 (42, 52) |
| 17(18)-EpETE | 3.6 (2.9, 5.4) | 3.8 (2.4, 6.0) | 3.5 (3.1, 4.9) | 3.7 (2.9, 7.1) | 4.4 (3.3, 5.4) | 4.5 (3.7, 7.0) | 4.2 (3.8, 6.6) | 3.6 (2.6, 6.0) |
| 19(20)-EpDPE | 9.6 (7.1, 11) | 9.7 (5.9, 12) | 9.2 (7.9, 10) | 10 (8.0, 13) | 10 (7.9, 11) | 11 (9.6, 15) | 11 (9.4, 14) | 8.9 (8.1, 12) |
| 14,15-DiHETrE | 1.3 (1.2, 1.5) | 1.3 (1.1, 1.6) | 1.3 (1.2, 1.4) | 1.3 (1.2, 1.7) | 1.4 (1.2, 1.6) | 1.6 (1.3, 2.3) | 1.4 (1.2, 1.9) | 1.4 (1.2, 1.5) |
| 17,18-DiHETE | 0.64 (0.34, 1.1) | 0.64 (0.38, 1.2) | 0.66 (0.39, 1.1) | 0.66 (0.31, 1.1) | 0.64 (0.39,1.2) | 0.72 (0.39, 1.2) | 0.60 (0.32, 1.3) | 0.65 (0.36, 1.2) |
| 19,20-DiHDPE | 2.8 (2.2, 4.0) | 3.0 (2.3, 4.1) | 2.9 (2.2, 4.1) | 2.8 (2.3, 4.3) | 3.1 (2.2, 4.1) | 3.2 (2.9, 4.1) | 3.2 (2.1, 4.2) | 3.0 (2.1, 4.3) |
| 5(*R,S*)-5-$F_{2t}$-IsoP | 0.37 (0.32, 0.41) | 0.40 (0.26, 0.48) | 0.33 (0.22, 0.41) | 0.37 (0.33, 0.46) | 0.37 (0.31, 0.47) | 0.36 (0.32, 0.54) | 0.42 (0.36, 0.54) | 0.44 (0.38, 0.57) |
| 9-HETE | 4.4 (3.6, 5.5) | 4.6 (3.8, 5.7) | 5.2 (4.4, 5.7) | 6.1 (5.0, 7.1) | 4.4 (4.2, 5.3) | 6.0 (4.8, 7.3) | 5.1 (4.4, 6.1) | 5.6 (4.7, 8.2) |
| 11-HETE | 3.0 (2.6, 3.6) | 3.1 (2.8, 3.5) | 3.4 (3.0, 4.3) | 5.0 (3.4, 5.9) | 6.4 (3.4, 11) | 7.7 (3.8, 16) | 8.3 (4.5, 14) | 13 (5.5, 18) |
| 18-HEPE | 0.72 (0.70, 1.3) | 0.82 (0.68, 1.4) | 0.83 (0.81, 1.5) | 1.1 (0.84, 1.8) | 1.0 (0.76, 1.6) | 1.1 (0.85, 2.2) | 1.3 (0.90, 2.3) | 2.1 (1.3, 3.0) |

* $PGE_2$ and $TxB_2$ are degraded during alkaline hydrolysis