# The distinct metabolism between large and small HDL indicates unique origins of human apolipoprotein A4

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**SUPPLEMENTAL MATERIAL**

Supplemental Figures 1, 2

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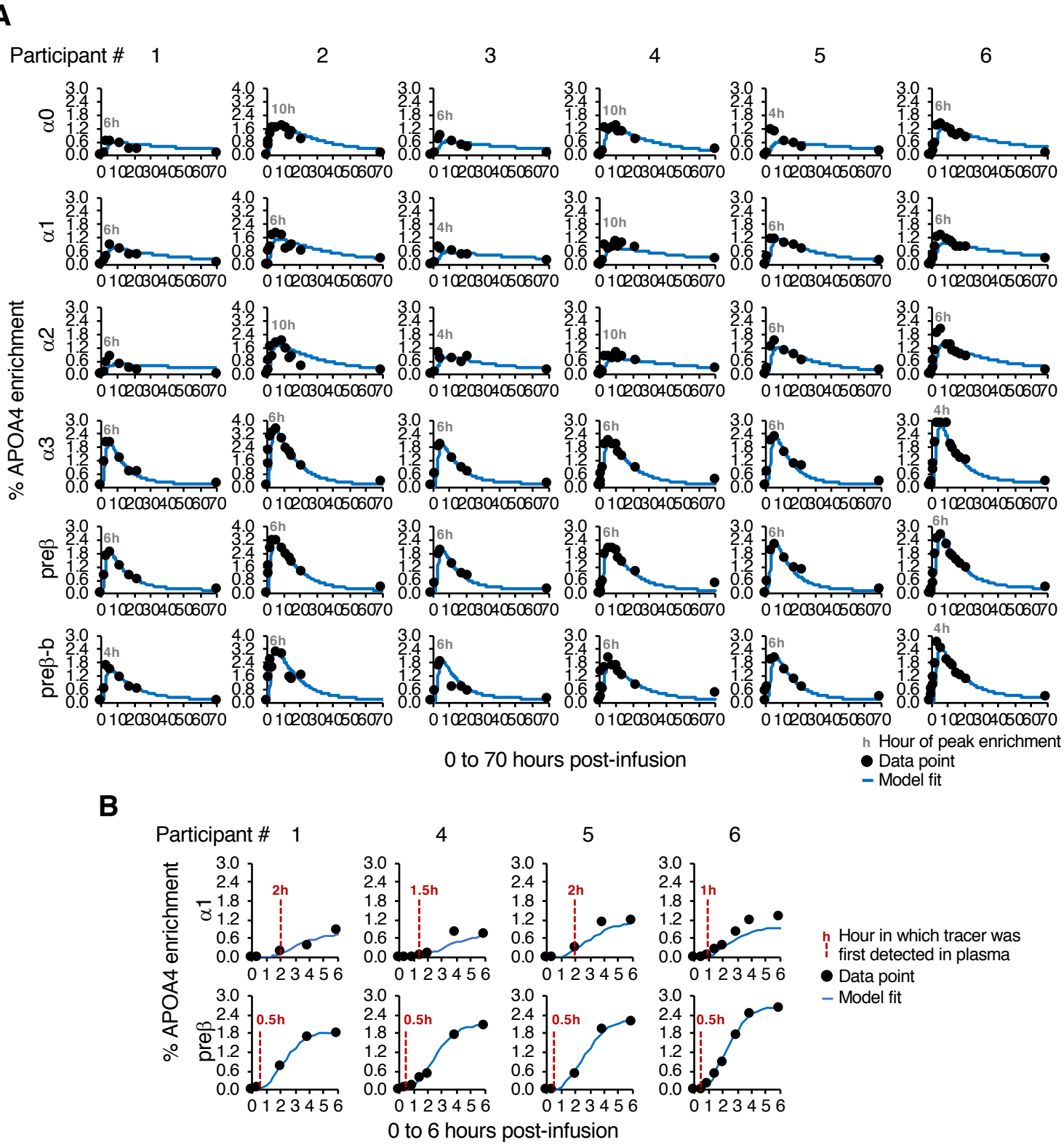
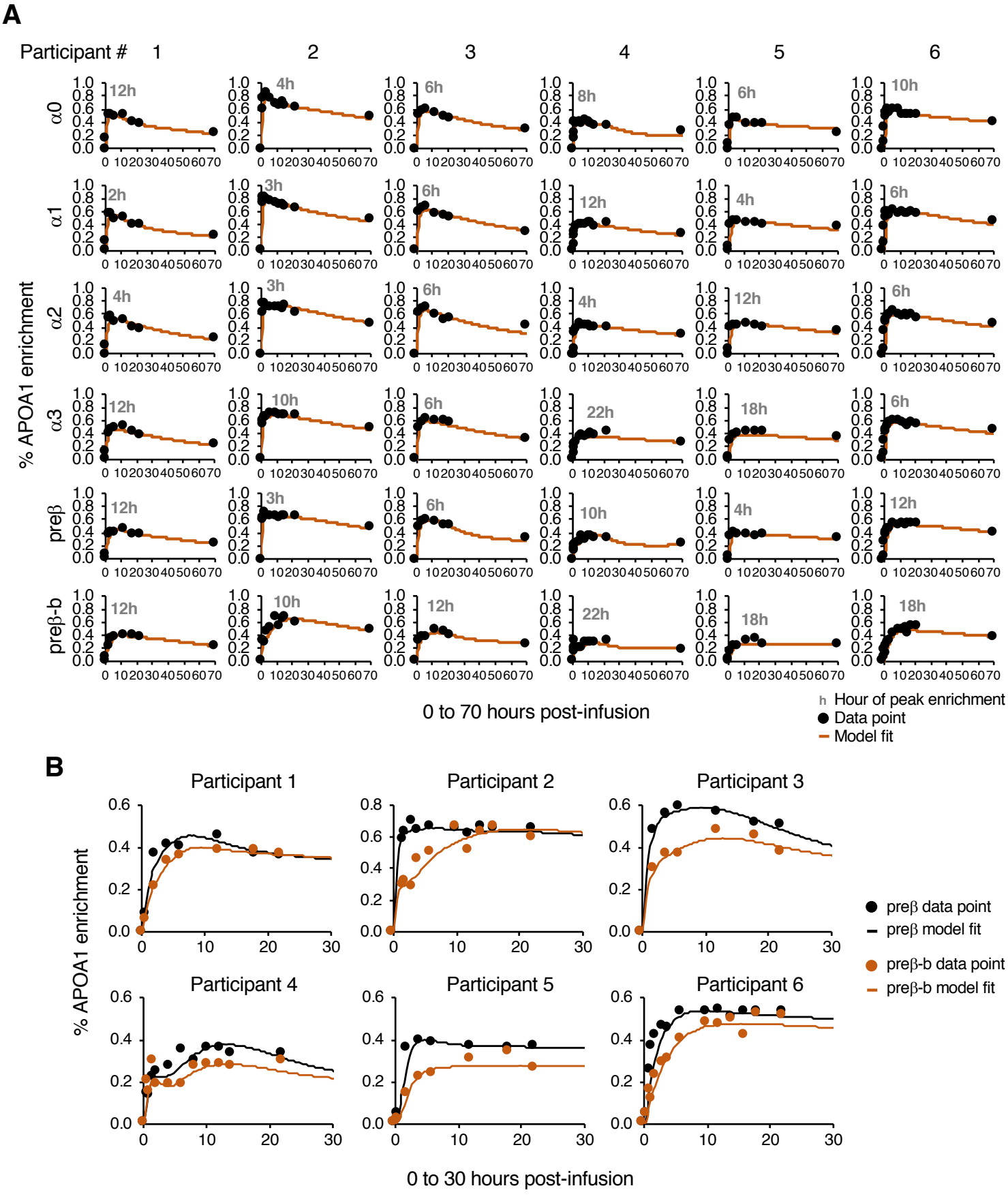
Supplemental Methods




**Supplemental Figure 1. APOA4 enrichment data and SAAM II-generated model fits for 6 participants.** (**A**) Enrichment data and model fits for each participant across the 6 HDL sizes over the full time course (0-70 hrs). Time point indicated in grey above each enrichment curve is the hour of peak enrichment for each size fraction. (**B**) Zoom into the first 6 hours of the time course. Dashed red line and hour indicate the time in which APOA4 tracer was first detected on large and small HDL in plasma (alpha1 and prebeta are shown as representative large and small sizes, respectively). Participants 2 and 3 are not shown since the 0.5 hour time point was not monitored in these participants (the earliest time point monitored for participants 2 and 3 was 1.5 hours and 2 hours, respectively).




**Supplemental Figure 2. APOA1 enrichment data and SAAM II-generated model fits for 6 participants.** (**A**) Enrichment data and model fits for each participant across the 6 HDL sizes. Time points indicated in grey above each enrichment curve is the hour of peak enrichment for each size fraction. (**B**) Zoomed in overlay comparing prebeta and prebeta-b model fits in the 6 participants.

| Participant ID# | Gender | Race | Age | Height (m) | IBW (kg) | Weight (kg) | BMI ($kg/m^2$) | TG (mg/dL) | Total-C (mg/dL) | LDL-C (mg/dL) | HDL-C (mg/dL) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1* | female | black | 33 | 1.70 | 72.5 | 85.8 | 29.6 | 50 | 142 | 95 | 37 |
| 2* | male | white | 39 | 1.85 | 85.9 | 110.3 | 32.1 | 138 | 176 | 103 | 45 |
| 3* | male | white | 49 | 1.73 | 74.8 | 94.2 | 31.5 | 262 | 158 | 82 | 24 |
| 4* | male | black | 48 | 1.75 | 76.9 | 91.7 | 29.8 | 64 | 171 | 116 | 42 |
| 5* | female | white | 25 | 1.69 | 71.3 | 75.6 | 26.5 | 54 | 132 | 73 | 48 |
| 6* | female | white | 40 | 1.68 | 70.4 | 86.1 | 30.6 | 139 | 219 | 147 | 44 |
| 7 | female | black | 25 | 1.58 | 62.1 | 87.2 | 35.1 | 62 | 145 | 85 | 48 |
| 8 | female | white | 23 | 1.58 | 62.1 | 68.8 | 27.7 | 87 | 123 | 59 | 47 |
| 9 | female | white | 70 | 1.65 | 68.3 | 85.5 | 31.3 | 72 | 193 | 125 | 54 |
| 10 | female | white | 62 | 1.60 | 64.1 | 90.5 | 35.3 | 76 | 175 | 105 | 55 |
| 11 | female | white | 33 | 1.61 | 65.2 | 80.8 | 31 | 88 | 193 | 122 | 53 |
| 12 | male | white | 59 | 1.83 | 83.8 | 92.8 | 27.7 | 59 | 144 | 96 | 36 |
| Mean (SD) | 8 female 4 male | 9 white 3 black | 42 (16) | 1.69 (0.09) | 71.4 (7.9) | 87.4 (10.3) | 30.7 (2.7) | 95.9 (60.1) | 164 (28.6) | 101 (24.5) | 44.4 (8.8) |

**Supplemental Table 1. Clinical characteristics.** BMI, body mass index; TG, triglyceride; total-C, total cholesterol, LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; *, participant samples included in the APOA1 and APOA4 metabolism analysis.

| Protein | Peptide sequence | *z* | Ion type | *m/z* | Label | Isolation *m/z* | RT (min) |
|---|---|---|---|---|---|---|---|
| APOA1 | THLAPYSDELR | 3 | precursor | 434.5543 | light M0 | 435.5606 | 8.5 to 10.5 |
| | THLAPYSDELR | 3 | precursor | 436.5669 | heavy M3 | | |
| | THL | 1 | b3 | 352.1979 | light M0 | | |
| | THL | 1 | b3 | 355.2168 | heavy M3 | | |
| | ELR | 1 | y3 | 417.2456 | light M0 | | |
| | ELR | 1 | y3 | 420.2644 | heavy M3 | | |
| | THLA | 1 | b4 | 423.2350 | light M0 | | |
| | THLA | 1 | b4 | 426.2539 | heavy M3 | | |
| | THLAP | 1 | b5 | 520.2878 | light M0 | | |
| | THLAP | 1 | b5 | 523.3066 | heavy M3 | | |
| | DELR | 1 | y4 | 532.2726 | light M0 | | |
| | DELR | 1 | y4 | 535.2914 | heavy M3 | | |
| | SDELR | 1 | y6 | 782.3679 | light M0 | | |
| | SDELR | 1 | y6 | 785.3867 | heavy M3 | | |
| | AKPALEDLR | 3 | precursor | 338.1977 | light M0 | 339.2040 | 7.5 to 9.5 |
| | AKPALEDLR | 3 | precursor | 340.2103 | heavy M3 | | |
| | LR | 1 | y2 | 288.2030 | light M0 | | |
| | LR | 1 | y2 | 291.2218 | heavy M3 | | |
| | EDLR | 1 | y4 | 532.2726 | light M0 | | |
| | EDLR | 1 | y4 | 535.2914 | heavy M3 | | |
| | VKDLATVYVDVLK | 3 | precursor | 488.2887 | light M0 | 489.2950 | 13 to 15 |
| | VKDLATVYVDVLK | 3 | precursor | 490.3013 | heavy M3 | | |
| | DVLK | 1 | y4 | 474.2922 | light M0 | | |
| | DVLK | 1 | y4 | 477.3111 | heavy M3 | | |
| | VDVLK | 1 | y5 | 573.3606 | light M0 | | |
| | VDVLK | 1 | y5 | 576.3795 | heavy M3 | | |
| | YVDVLK | 1 | y6 | 736.4240 | light M0 | | |
| | YVDVLK | 1 | y6 | 739.4428 | heavy M3 | | |
| | VKDLAT | 1 | b6 | 628.3665 | light M0 | | |
| | VKDLAT | 1 | b6 | 631.3853 | heavy M3 | | |
| APOA4 | LVPFATELHER | 3 | precursor | 437.9067 | light M0 | 438.9130 | 11 to 13 |
| | LVPFATELHER | 3 | precursor | 439.9193 | heavy M3 | | |
| | LHER | 1 | y4 | 554.3045 | light M0 | | |
| | LHER | 1 | y4 | 557.3234 | heavy M3 | | |
| | ELHER | 1 | y5 | 683.3471 | light M0 | | |
| | ELHER | 1 | y5 | 686.3659 | heavy M3 | | |
| | TELHER | 1 | y6 | 784.3948 | light M0 | | |
| | TELHER | 1 | y6 | 787.4136 | heavy M3 | | |
| | ATELHER | 2 | y7 | 428.2196 | light M0 | | |
| | ATELHER | 2 | y7 | 429.7290 | heavy M3 | | |
| | FATELHER | 2 | y8 | 501.7538 | light M0 | | |
| | FATELHER | 2 | y8 | 503.2632 | heavy M3 | | |
| | ISASAEELR | 2 | precursor | 488.2589 | light M0 | 489.0136 | 8 to 10 |
| | ISASAEELR | 2 | precursor | 489.7683 | heavy M3 | | |
| | ELR | 1 | y3 | 417.2456 | light M0 | | |
| | ELR | 1 | y3 | 420.2644 | heavy M3 | | |
| | EELR | 1 | y4 | 546.2882 | light M0 | | |
| | EELR | 1 | y4 | 549.3070 | heavy M3 | | |
| | SAEELR | 1 | y6 | 704.3573 | light M0 | | |
| | SAEELR | 1 | y6 | 707.3762 | heavy M3 | | |
| | ALVQQMEQLR | 2 | precursor | 608.3293 | light M0 | 609.8388 | 11.5 to 13.5 |
| | ALVQQMEQLR | 2 | precursor | 611.3482 | heavy M3 | | |
| | QLR | 1 | y3 | 416.2616 | light M0 | | |
| | QLR | 1 | y3 | 419.2804 | heavy M3 | | |
| | EQLR | 1 | y4 | 545.3042 | light M0 | | |
| | EQLR | 1 | y4 | 548.3230 | heavy M3 | | |
| | MEQLR | 1 | y5 | 676.3447 | light M0 | | |
| | MEQLR | 1 | y5 | 679.3635 | heavy M3 | | |
| | QMEQLR | 1 | y6 | 804.4032 | light M0 | | |
| | QMEQLR | 1 | y6 | 807.4221 | heavy M3 | | |

**Supplemental Table 2. APOA1 and APOA4 peptides used to quantify tracer enrichment by targeted mass spectrometry.** *z,* charge. *m/z*, mass-to-charge of the light M0 (D0-Leu) and heavy M3 (D3-Leu) fragment ions. Isolation *m/z*, the average mass of the light and heavy ions used as the center of the 4 Da targeted isolation window. RT, retention time range for each peptide.

| | | APOA4 | | | APOA1 | | |
|---|---|---|---|---|---|---|---|
| HDL size | Participant # | FCR (pools/day) | PR (mg/kg/day) | PS (mg) | FCR (pools/day) | PR (mg/kg/day) | PS (mg) |
| α0 | 1 - female | 0.60 | 0.003 | 0.42 | 4.48 | 2.08 | 41.24 |
| | 2 - male | 1.02 | 0.066 | 7.09 | 3.22 | 1.07 | 36.61 |
| | 3 - male | 0.68 | 0.006 | 0.74 | 0.99 | 0.32 | 29.62 |
| | 4 - male | 1.05 | 0.049 | 4.05 | 3.94 | 1.56 | 34.70 |
| | 5 - female | 0.54 | 0.007 | 0.94 | 10.13 | 1.83 | 13.24 |
| | 6 - female | 0.87 | 0.018 | 1.69 | 4.07 | 1.28 | 26.29 |
| | mean (SD) | 0.79 (0.22) | 0.025 (0.026) | 2.49 (2.61) | 4.47 (3.04) | 1.36 (0.62) | 30.28 (9.86) |
| | *P*-value | 0.19 | 0.22 | 0.25 | 0.21 | 0.17 | 0.50 |
| α1 | 1 - female | 0.84 | 0.010 | 1.06 | 2.12 | 14.37 | 601.89 |
| | 2 - male | 0.87 | 0.058 | 7.37 | 1.66 | 7.50 | 495.50 |
| | 3 - male | 0.62 | 0.011 | 1.64 | 0.57 | 2.89 | 459.72 |
| | 4 - male | 0.58 | 0.033 | 4.98 | 0.55 | 1.70 | 272.80 |
| | 5 - female | 1.10 | 0.070 | 4.65 | 0.29 | 3.67 | 921.38 |
| | 6 - female | 0.67 | 0.035 | 4.45 | 0.47 | 3.59 | 639.18 |
| | mean (SD) | 0.78 (0.20) | 0.036 (0.024) | 4.02 (2.33) | 0.94 (0.76) | 5.62 (4.71) | 565.08 (216.87) |
| | *P*-value | 0.32 | 0.85 | 0.57 | 0.96 | 0.49 | 0.07 |
| α2 | 1 - female | 0.46 | 0.004 | 0.82 | 0.65 | 13.03 | 1771.71 |
| | 2 - male | 1.00 | 0.058 | 6.40 | 0.33 | 7.60 | 2514.78 |
| | 3 - male | 0.90 | 0.026 | 2.68 | 1.02 | 19.24 | 1709.82 |
| | 4 - male | 0.55 | 0.017 | 2.74 | 0.30 | 3.61 | 1058.91 |
| | 5 - female | 1.19 | 0.056 | 3.44 | 0.44 | 16.74 | 2754.21 |
| | 6 - female | 0.97 | 0.077 | 6.60 | 0.36 | 10.55 | 2435.12 |
| | mean (SD) | 0.84 (0.28) | 0.040 (0.028) | 3.78 (2.28) | 0.52 (0.28) | 11.80 (5.79) | 2040.76 (637.99) |
| | *P*-value | 0.83 | 0.67 | 0.89 | 0.82 | 0.57 | 0.34 |
| α3 | 1 - female | 2.92 | 0.25 | 7.70 | 0.56 | 8.40 | 1341.32 |
| | 2 - male | 2.32 | 1.10 | 52.03 | 0.30 | 6.17 | 2249.77 |
| | 3 - male | 2.83 | 0.59 | 18.94 | 0.54 | 7.81 | 1324.46 |
| | 4 - male | 1.94 | 0.76 | 34.39 | 0.23 | 9.74 | 3688.55 |
| | 5 - female | 2.69 | 0.92 | 25.12 | 0.24 | 6.84 | 2106.91 |
| | 6 - female | 2.46 | 3.70 | 125.68 | 0.34 | 6.30 | 1559.61 |
| | mean (SD) | 2.52 (0.37) | 1.22 (1.25) | 43.98 (42.73) | 0.37 (0.14) | 7.54 (1.38) | 2045.11 (894.09) |
| | *P*-value | 0.34 | 0.52 | 0.68 | 0.88 | 0.59 | 0.39 |
| preβ | 1 - female | 2.52 | 5.53 | 195.07 | 3.52 | 7.39 | 187.04 |
| | 2 - male | 2.12 | 8.11 | 420.10 | 5.96 | 5.49 | 101.19 |
| | 3 - male | 2.83 | 7.89 | 253.43 | 1.40 | 0.49 | 32.05 |
| | 4 - male | 1.94 | 5.98 | 271.21 | 12.61 | 7.69 | 53.59 |
| | 5 - female | 2.64 | 15.05 | 417.43 | 2.76 | 1.72 | 45.64 |
| | 6 - female | 2.24 | 17.47 | 651.94 | 1.64 | 1.77 | 89.69 |
| | mean (SD) | 2.38 (0.34) | 10.01 (5.01) | 368.20 (166.24) | 4.65 (4.23) | 4.09 (3.16) | 84.86 (56.63) |
| | *P*-value | 0.61 | 0.28 | 0.51 | 0.34 | 0.76 | 0.41 |
| preβ-b | 1 - female | 1.98 | 0.014 | 0.61 | 3.84 | 3.60 | 83.51 |
| | 2 - male | 2.12 | 0.035 | 1.79 | 13.97 | 1.24 | 9.74 |
| | 3 - male | 2.74 | 0.026 | 0.86 | 1.33 | 0.15 | 9.94 |
| | 4 - male | 1.56 | 0.029 | 1.63 | 18.28 | 4.18 | 20.10 |
| | 5 - female | 2.39 | 0.033 | 1.02 | 0.17 | 0.04 | 17.63 |
| | 6 - female | 2.02 | 0.030 | 1.22 | 0.28 | 0.05 | 15.54 |
| | mean (SD) | 2.14 (0.40) | 0.028 (0.008) | 1.19 (0.45) | 6.31 (7.84) | 1.54 (1.88) | 26.08 (28.44) |
| | *P*-value | 0.98 | 0.56 | 0.24 | 0.19 | 0.73 | 0.37 |
| system | 1 - female | 2.52 | 5.82 | 205.68 | 0.65 | 29.47 | 4026.70 |
| | 2 - male | 2.09 | 9.42 | 494.78 | 0.33 | 16.17 | 5407.59 |
| | 3 - male | 2.79 | 8.54 | 278.29 | 0.59 | 22.95 | 3565.61 |
| | 4 - male | 1.89 | 6.86 | 319.00 | 0.28 | 16.32 | 5128.66 |
| | 5 - female | 2.61 | 16.14 | 452.60 | 0.28 | 22.24 | 5859.01 |
| | 6 - female | 2.25 | 21.34 | 791.58 | 0.36 | 20.37 | 4765.42 |
| | mean (SD) | 2.36 (0.34) | 11.35 (6.08) | 423.65 (210.17) | 0.41 (0.16) | 21.25 (4.95) | 4792.17 (862.69) |
| | *P*-value | 0.55 | 0.31 | 0.57 | 0.85 | 0.20 | 0.83 |

**Supplemental Table 4. Kinetic parameters of APOA1 and APOA4 across 6 HDL sizes.** FCR, fractional catabolic rate; PR, production rate; PS, pool size. *P*-value, kinetic parameters for each protein in each HDL size were compared between females and males by two-tailed, unpaired *t*-test (with unequal variance).

| Gene name | UniProt entry # | Protein name | Average protein abundance (mean ± SD) for each protein detected in preβ-b (n=1 to 12 participants) |
|---|---|---|---|
| IGHM | P01871-2 | Isoform 2 of Ig mu chain C region | - |
| FN1 | P02751; P02751-15 | Fibronectin | 262,000 ± 145,434 (n=10) |
| A2M | P01023 | Alpha-2-macroglobulin | 892,500 ± 571,882 (n=8) |
| CFH | P08603 | Complement factor H | - |
| APOB | P04114 | Apolipoprotein B-100 | 210,000 (n=1) |
| C1QC | P02747 | Complement C1q subcomponent subunit C | 5,411,111 ± 1,678,871 (n=9) |
| LGALS3BP | Q08380 | Galectin-3-binding protein | - |
| CLU | P10909-2 | Clusterin | 5,355,454 ± 2,052,760 (n=11) |
| FGB | P02675 | Fibrinogen beta chain | 1,082,727 ± 497,555 (n=11) |
| FGG | C9JC84 | Fibrinogen gamma chain | 17,883,333 ± 14,348,571 (n=12) |
| IGLC2 | P0CG05 | Ig lambda-2 chain C regions | 520,000 (n=1) |
| FGA | P02671 | Fibrinogen alpha chain | 23,833,333 ± 14,529,489 (n=12) |
| HP | P00738 | Haptoglobin | 743,333 ± 718,850 (n=6) |
| HBB | P68871 | Hemoglobin subunit beta | 300,000 (n=1) |
| C1R | P00736 | Complement C1r subcomponent | 556,666 ± 332,014 (n=3) |
| APOL1 | O14791-2 | Apolipoprotein L1 | 722,727 ± 773,266 (n=11) |
| PLTP | B3KUE5 | Phospholipid transfer protein | - |
| APOM | O95445 | Apolipoprotein M | - |
| APOE | P02649 | Apolipoprotein E | 14,649,166 ± 11,707,895 (n=12) |
| ITIH2 | P19823 | Inter-alpha-trypsin inhibitor heavy chain H2 | 790,000 ± 181,934 (n=3) |
| C3 | P01024 | Complement C3 | 2,640,000 ± 1,521,578 (n=12) |
| GPLD1 | P80108 | Phosphatidylinositol-glycan-specific phospholipase D | - |
| CETP | P11597 | Cholesteryl ester transfer protein | - |
| SAA4 | P35542 | Serum amyloid A-4 protein | 782,500 ± 156,677 (n=12) |
| APOA2 | V9GYM3 | Apolipoprotein A-II | 6,836,363 ± 4,517,139 (n=11) |
| APCS | P02743 | Serum amyloid P-component | - |
| CFI | E7ETH0 | Complement factor I light chain | - |
| APOA1 | P02647 | Apolipoprotein A-I | 424,283,333 ± 385,245,446 (n=12) |
| APOC4-APOC2 | K7ER74 | Protein APOC4-APOC2 | - |
| AGT | P01019 | Angiotensinogen | - |
| VTN | P04004 | Vitronectin | 3,875,454 ± 2,872,181 (n=11) |
| APOD | C9JF17 | Apolipoprotein D | 5,566,666 ± 3,148,255 (n=12) |
| APOC3 | B0YIW2 | Apolipoprotein C-III | 7,133,333 ± 8,831,336 (n=9) |
| PON1 | F5H4W9; P27169 | Serum paraoxonase/arylesterase 1 | 257,333,333 ± 192,957,995 (n=12) |
| C4B | P0C0L5 | Complement C4-B | 2,700,000 ± 863,133 (n=5) |
| CNDP1 | Q96KN2 | Beta-Ala-His dipeptidase | - |
| TF | P02787 | Serotransferrin | - |
| SERPINA3 | P01011 | Alpha-1-antichymotrypsin | 755,000 ± 629,325 (n=2) |
| PROS1 | P07225 | Vitamin K-dependent protein S | 320,000 ± 69,282 (n=4) |
| ITIH4 | Q14624 | Inter-alpha-trypsin inhibitor heavy chain H4 | - |
| PLXDC2 | Q6UX71 | Plexin domain-containing protein 2 | 460,000 (n=1) |
| KNG1 | P01042-2; P01042 | Kininogen-1 | 792,000 ± 411,424 (n=5) |
| HPX | P02790 | Hemopexin | - |
| SERPINC1 | P01008 | Antithrombin-III | 580,000 (n=1) |
| C1S | P09871 | Complement C1s subcomponent | - |
| LCAT | P04180 | Phosphatidylcholine-sterol acyltransferase | - |
| CDH5 | P33151 | Cadherin-5 | 1,000,000 (n=1) |
| A1BG | P04217 | Alpha-1B-glycoprotein | - |
| F5 | P12259 | Coagulation factor V | 560,000 ± 254,990 (n=11) |
| AHSG | C9JV77 | Alpha-2-HS-glycoprotein | - |
| SERPINA1 | P01009 | Alpha-1-antitrypsin | 4,768,181 ± 6,644,517 (n=11) |
| APOA4 | P06727 | Apolipoprotein A-IV | 13,566,666 ± 8,804,062 (n=12) |
| ALB | P02768 | Serum albumin | 8,625,000 ± 3,031,388 (n=12) |

**Supplemental Table 7. HDL proteins detected in prebeta-b.** List of 53 proteins identified on HDL in 12 participants. The horizontal black lines separate proteins in the 5 protein clusters. The order in which the proteins are listed corresponds with **Figure 6A**. The right most column highlights the average abundance of prebeta-b proteins that were detected in 1 to 12 participants. The prebeta-b proteins detected in 9 to 12 participants in this list correspond with the proteins shown in **Figure 6B,C**. (n=), indicates the number of participates in which each protein was identified; -, HDL proteins not detected in prebeta-b.

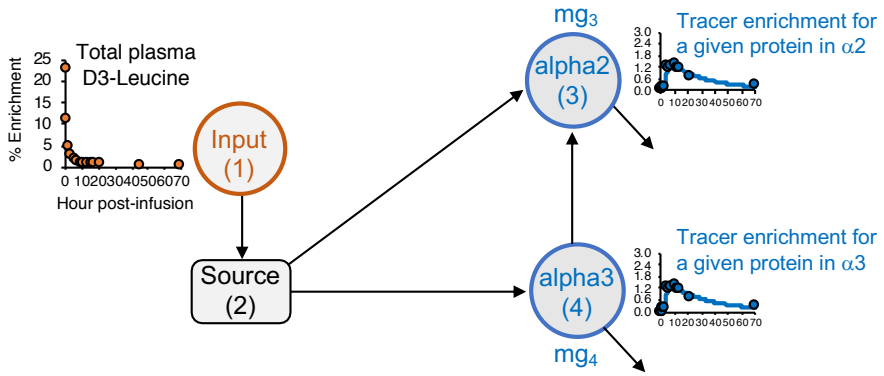
## SUPPLEMENTAL METHODS

### Overview of compartmental modeling

The following section provides an overview of the compartmental modeling process used to determine the metabolism and kinetic parameters of HDL proteins across one or more HDL sizes in humans using SAAM II modeling software (The Epsilon Group, http://tegvirginia.com).

**Model components – compartments and arrows**: The basic structure of a compartmental model used to describe the metabolism of a given protein on a given HDL size typically contains 3 or more compartments - an input, source, and one or more HDL size compartments. An example of this basic model structure with 2 HDL sizes is shown below in **Supplemental Methods Figure 1**. Each compartment in the model represents a different physiological system or process. The input compartment (compartment 1) represents the plasma amino acid precursor pool. The total plasma D3-Leucine tracer enrichment data for a given participant is uploaded to this compartment and expressed as a forcing function that drives the appearance of D3-Leucine tracer in the model. The source compartment (compartment 2) accounts for the time it takes for labeled protein to appear on each HDL size in plasma. It represents the site of synthesis and secretion of the protein, and potentially any intermediate transfer steps (such as transfer to and from an APOB-containing lipoprotein) before it enters a given HDL size in plasma. The source compartment can be represented in the model as either a single compartment or a multi-compartment delay, and can be optimized to fit the time of appearance and ascending slope of the tracer-labeled protein as it appears on the HDL sizes in circulation. Compartments 3 and 4 represent alpha2 and alpha3 HDL, respectively, in plasma. Protein tracer enrichment data across the full metabolic study time course (i.e., 0-70 hours post-infusion in the present study) and pool size data are incorporated into each HDL size compartment (**Supplemental Methods Figure 1**). The pool size of a given protein in alpha2 and alpha3 are shown in the model as $mg_3$ and $mg_4$, respectively (**Supplemental Methods Figure 1**).

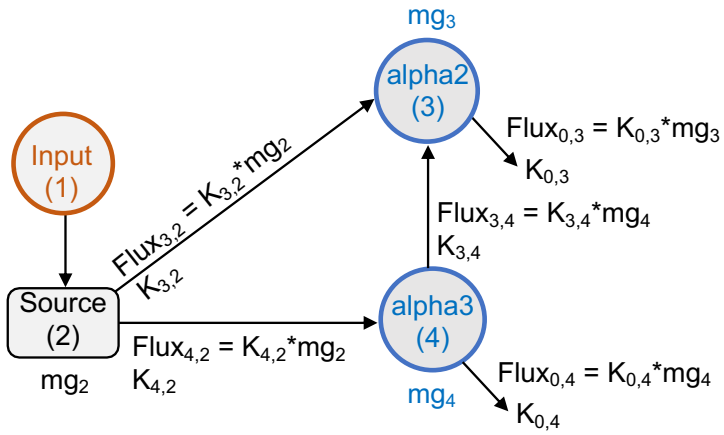
Pathways of tracer movement between and out of compartments are represented by arrows. Arrows from the source compartment into each HDL size represent the production of a given protein onto that HDL size. Arrows between compartments represent transfer of protein from one HDL size to another, and downward arrows represent protein removal out of that HDL size in circulation, such as by hepatic uptake or by protein transfer to a compartment not measured in the study (i.e., APOB-containing lipoproteins).




**Supplemental Methods Figure 1. Example structure of a compartmental model used to determine the metabolism of a given HDL protein on specific HDL sizes in plasma**. Circles and squares represent compartments, and numbers inside the compartment represent the compartment number. Total plasma D3-Leucine enrichment is uploaded to the input compartment (compartment 1, orange). Tracer enrichment and pool size data for a given HDL protein in a given HDL size are uploaded to the corresponding HDL size compartment (alpha2, compartments 3; alpha3, compartment 4; blue). $Mg_x$, the pool size in milligrams of a given protein in compartment x ($mg_3$, alpha2 pool size; $mg_4$, alpha3 pool size).

**Model-generated rate constants and flux values**: SAAM II uses the tracer and pool size data to generate a system of differential equations to estimate the rate and amount of protein entering and leaving each compartment per day for each pathway. A rate constant and flux value are estimated for each pathway (**Supplemental Methods Figure 2**). Rate constants ($K_{x,y}$) represent the rate at which a given protein is transferring into compartment x from compartment y in pools/day. For example, $K_{3,4}$ represents the rate at which a given protein is transferring to compartment 3 from compartment 4. $K_{0,y}$ represents the rate at which a given protein is being removed out of the model system (0) from compartment y. Flux values ($Flux_{x,y}$) indicate the amount of protein in milligrams (mg) transferring into compartment x from compartment y per day, and are calculated by taking the rate constant for that pathway ($K_{x,y}$) times the pool size of the protein in compartment y: $Flux_{x,y} = K_{x,y} * mg_y$. For instance, the flux of protein into

compartment 3 from compartment 4 and the flux of protein out of compartment 3 are equal to $Flux_{3,4} = K_{3,4}*mg_4$ and $Flux_{0,3} = K_{0,3}*mg_3$, respectively.

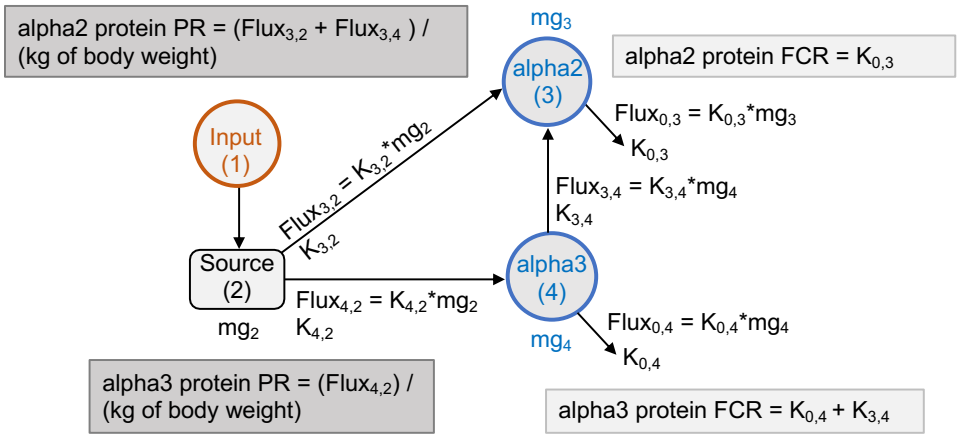



**Supplemental Methods Figure 2. Model-generated rate constants and flux values for each pathway**. $K_{x,y}$, rate of protein transfer into compartment x from compartment y by a given pathway (pools/day). $Mg_x$, the pool size in milligrams of protein in compartment x. $Flux_{x,y}$, amount of protein (mg) entering compartment x from compartment y by a given pathway per day (mg/day).

For these studies, we assume that the given HDL protein being modeled is in steady-state: the amount of protein entering a given HDL size per day is equal to the amount of that protein leaving that size, and the protein pool size in each compartment remains constant. Based on this steady-state premise, the flux of protein entering a given compartment must equal the flux of protein leaving the compartment. For example, in **Supplemental Methods Figure 2**, the flux of protein into alpha3 from the source ($Flux_{4,2}$) is equal to the flux of protein leaving alpha3 ($Flux_{0,4} + Flux_{3,4}$), and the flux of protein into alpha2 ($Flux_{3,2} + Flux_{3,4}$) is equal to the flux of protein leaving alpha2 ($Flux_{0,3}$).

**Calculating kinetic parameters**: The model-generated rate constants and fluxes are then used to calculate the fractional catabolic rate (FCR) and production rate for a given HDL protein on each HDL size. The FCR is the fraction of a given plasma protein pool turned over per day and is determined for each protein in each HDL size by taking the sum of the rate constants exiting that compartment. In **Supplemental Methods Figure 3**, the protein FCR out of alpha2 is equal to $K_{0,3}$ and the protein FCR out of alpha3 is equal to $K_{0,4} + K_{3,4}$ (light grey boxes). The production rate is the amount (mg) of protein produced or transferred into each HDL size per day per kg of body weight and is determined for a given protein on each HDL size by taking the sum of the fluxes into a given HDL size compartment divided by body weight. In **Supplemental Methods Figure 3**, the protein production rate into alpha3 is equal to

($Flux_{4,2}$ / kg of body weight), and the production rate into alpha2 is equal to ([$Flux_{3,2}$ + $Flux_{3,4}$] / kg of body weight) (dark grey boxes). Given that the system is in steady-state, and the flux of protein coming into each HDL size is equal to the flux of protein leaving that size, the production rate can also be calculated using the protein FCR and pool size for a given HDL size: Production rate = FCR (pools/day) x pool size (mg) / body weight (kg).

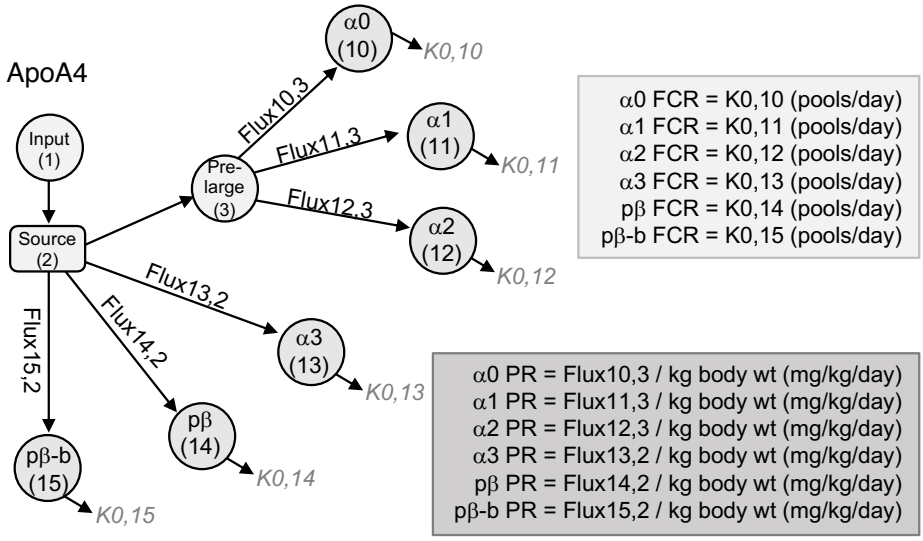



**Supplemental Methods Figure 3. Using model-generated rate constants and flux values to determine the FCR and production rate of a given HDL protein on each HDL size**. The FCR for a given protein out of each HDL size is determined by taking the sum of all rate constants exiting that size (pools/day, light grey boxes). The production rate (PR) for a given protein into each HDL size is determined by taking the sum of the flux values into each HDL size divided by body weight (mg/kg/day, dark grey boxes).

In addition to calculating the protein FCR and production rate out of and into each HDL size, respectively, we also determine the system FCR and the system production rate. The system FCR is the fraction of protein in the entire model system turned over per day (pools/day), and is calculated by taking the system production rate (mg/kg/day) divided by the system pool size (total mg of protein in all HDL sizes) x body weight (kg). The system production rate (mg/kg/day) is the total amount of protein (mg) entering the model system per day per kg of body weight, and is determined by taking the sum of all fluxes (mg/day) exiting the source compartment divided by body weight (kg).

**APOA4 model and kinetic parameters**: The basic compartmental model structure and principles outlined above were used to generate the APOA4 compartmental model and calculate the APOA4 kinetic parameters. **Supplemental Methods Figure 4** shows the APOA4 compartmental model

and highlights the rate constants and flux values used to calculate the FCR and production rate, respectively, of APOA4 on each HDL size.




**Supplemental Methods Figure 4**. APOA4 compartmental model and the rate constants and flux values used to calculate FCR and production rate (PR), respectively, for APOA4 on each HDL size fraction in plasma.

**APOA1 model and kinetic parameters**: The basic compartmental model structure and principles outlined above were used to generate the APOA1 compartmental model and calculate the APOA1 kinetic parameters. A detailed description of APOA1 model development has been described previously (Mendivil et al. Arterioscler Thromb Vasc Biol. 2016).

## REFERENCES

Mendivil CO, et al. Novel pathways of apolipoprotein A-I metabolism in high-density lipoprotein of different sizes in humans. *Arterioscler Thromb Vasc Biol.* 2016:57(4):714-28.