SUPPLEMENTAL INFORMATION:

Unbound Free Fatty Acid Profiles in Human Plasma and the Unexpected Absence of Unbound Palmitoleate.

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Supplemental Table S1: Calibration parameters for the probes of Table2 and the 9 FFAu of Table 1. KQ, in nM, is the product of the probe FFAu binding constant (Kd) and Q as defined in equation 1.

Probe		MA		PA		POA		SA		OA	
ID	Ro	K_dQ	Rm	K_dQ	Rm	K_dQ	Rm	K_dQ	Rm	K_dQ	Rm
L2P22G6	0.221	44.8	1.57	25.4	1.79	112	1.47	35.5	0.89	62.9	1.25
L2P22G6K27C	0.217	177.1	2.88	31.4	1.15	93.2	1.10	9.0	0.50	14.3	0.44
L4BP4B9	0.234	43.6	6.12	28.9	4.03	205	6.93	52.7	1.27	229	4.23
L10P7A4-L30C	0.259	13.8	0.22	4.5	0.30	13.6	0.73	5.0	0.53	5.4	1.34
L11P7B3-V26C	0.193	123	2.45	18.7	4.11	105	3.29	3.6	4.53	12.2	4.72
L13EP16E11	0.141	303	0.30	51.7	0.45	66.2	0.46	16.1	0.49	7.46	0.60
L19CP10C7	0.406	869	0.81	44.7	1.82	52.9	1.76	11.0	2.05	8.64	2.14
L36P15B7	0.116	12671	4.62	46.5	0.24	56.8	0.51	15.1	0.31	8.77	0.63
L50BP4E2	0.082	113.5	4.64	134	5.70	639	4.59	65.4	0.59	851	3.21
L50BP9D5	0.356	23.2	5.03	21.1	4.70	21	5.21	132.	3.62	87.1	5.54
L50AP31A2	0.142	18.6	1.12	49.0	0.90	289	1.78	128.	0.43	2093	3.88
L61P8B12	0.053	430.2	4.72	54.0	8.26	37.8	9.59	40.7	2.71	37.8	8.27
L71BP33D1	0.105	1642.7	0.35	44.2	0.21	69.5	0.39	2.1	0.41	10.2	0.57
L76P9E4	0.028	63.9	0.05	6.7	0.09	37.3	0.31	4.0	0.28	5.21	0.75
L106P3B10	0.453	26.9	2.78	64.4	1.39	267	2.43	50.5	0.67	92.8	1.10
L118P4H5	0.081	1879	0.44	194	0.36	972	0.49	77.1	0.20	280	0.92
L119P3E5	0.230	70.6	2.43	32.5	3.50	115	2.13	29.6	1.44	58.4	2.05
L127P3G2	0.096	1494	0.13	235	0.16	687	0.17	174.4	0.33	109	0.40
L138P1H8N24C	0.567	247.1	4.83	53.5	4.91	492	5.52	39.6	3.14	27.9	4.81
L156P3F926C	0.067	80.3	1.64	5.9	1.02	2.5	3.45	4.8	1.91	2.83	2.79

Supplemental Table S1:continued

Probe ID	Ro	LA K_dQ	LA Rm	LNA K_dQ	LNA Rm	AA K_dQ	AA Rm	DHA K_dQ	DHA Rm
L2P22G6	0.221	143	1.43	307	1.53	526	1.46	730	1.06
L2P22G6K27C	0.217	76	0.85	146	1.05	64	0.59	81	0.45
L4BP4B9	0.234	1064	10.44	1156	8.48	950	4.60	429	3.95
L10P7A4-L30C	0.259	8	1.94	20	1.67	12	2.98	18	3.96
L11P7B3-V26C	0.193	67	4.68	145	3.48	65	4.81	73	4.35
L13EP16E11	0.141	29	0.60	138	0.68	18	0.53	34	0.50
L19CP10C7	0.406	8	2.75	13	2.20	31	2.28	36	1.75
L36P15B7	0.116	27	1.25	83	1.26	7	2.65	3	3.36
L50BP4E2	0.082	989	3.84	2103	4.72	3307	3.78	18055	3.88
L50BP9D5	0.356	80	5.94	177	5.88	227	5.09	891	4.09
L50AP31A2	0.142	9070	10.20	698	0.92	939	0.85	56003	10.20
L61P8B12	0.053	144	7.07	344	8.24	239	2.60	328	1.51
L71BP33D1	0.105	14	0.58	48	0.53	2	0.78	3	0.79
L76P9E4	0.028	19	0.78	73	0.50	26	0.66	31	0.80
L106P3B10	0.453	190	1.35	385	1.67	194	0.96	270	0.99
L118P4H5	0.081	731	1.16	1725	1.22	622	2.06	238	3.72
L119P3E5	0.230	192	3.27	370	3.22	340	2.33	558	1.49
L127P3G2	0.096	312	0.71	861	0.54	11	1.14	39	1.00
L138P1H8N24C	0.567	44	3.74	16	5.03	177	2.37	216	3.99
L156P3F926C	0.067	17	3.80	38	3.83	39	2.10	151	4.11

Supplemental Table S2: Average R values and CVs for NIST plasma measured at 15, 30, 45 and 60 minutes. These are the averages of the R values used to obtain the FFAu profiles in Table 6. The average CV for all the probes and measurements is 0.45%, well within the Table 8 predicted CV needed to obtain reliable FFAu profiles for samples with total FFAu >1.5 nM.

	R	SD	CV
L2P22	0.2321	0.0006	0.26%
L4BP4B9	0.2687	0.0021	0.77%
L10P7A4 30C	0.3094	0.0008	0.26%
L11P7B3 26C	0.3022	0.0029	0.95%
L19CP10C7	0.4681	0.0012	0.26%
L36P15B7-1	0.1546	0.0007	0.42%
L50AP31A2	0.1520	0.0004	0.24%
L50BP4E2	0.0911	0.0002	0.26%
L50BP9D5	0.4187	0.0006	0.13%
L61P8B12	0.1287	0.0015	1.17%
L71BP33D1	0.1315	0.0002	0.19%
L76P9E4	0.0589	0.0007	1.12%
L106P3B10	0.4641	0.0015	0.31%
L119P3E5	0.2513	0.0011	0.44%
L127P3G2	0.1002	0.0003	0.34%
L138P1H8 N24C	0.5884	0.0025	0.43%
L156P3F9 26C	0.4821	0.0039	0.80%
L118P4H5	0.0810	0.0002	0.27%
L13EP16E11	0.1479	0.0002	0.13%
L2P22 K27C	0.2354	0.0005	0.21%
Average CV			**0.45%**

Supplemental Table S3: Response profiles for each of the 20 probes of table 2. The probe response profiles were calculated using equation (3) for each probe and with the 9 FFAu set to 1 nM. For most of the probes there is generally one FFAu for which the response is largest and such a probe is considered specific for that FFAu because it may provide a substantial constraint on that FFAu's concentration derived from the fitting algorithm (equations (4)).

	L2P22G6	**L2P22G6K27C**	**L4BP4B9**	**L10P7A4-L30C**	**L11P7B3-V26C**
MA	0.133	0.069	0.563	-0.011	0.094
PA	0.270	0.133	0.541	0.026	1.030
POA	0.050	0.043	0.139	0.125	0.152
SA	0.083	0.131	0.082	0.175	4.873
OA	0.073	0.066	0.074	0.654	1.779
LA	0.038	0.038	0.041	0.706	0.342
LNA	0.019	0.026	0.030	0.256	0.117
AA	0.011	0.026	0.020	0.784	0.361
DHA	0.005	0.013	0.037	0.746	0.292
	L13EP16E11	**L19CP10C7**	**L36P15B7**	**L50BP4E2**	**L50BP9D5**
MA	0.004	0.001	0.003	0.484	0.543
PA	0.042	0.076	0.023	0.504	0.550
POA	0.033	0.062	0.059	0.085	0.621
SA	0.146	0.337	0.106	0.093	0.069
OA	0.389	0.442	0.454	0.045	0.165
LA	0.107	0.619	0.345	0.046	0.195
LNA	0.028	0.312	0.117	0.027	0.087
AA	0.142	0.145	2.782	0.014	0.058
DHA	0.072	0.089	7.810	0.003	0.012

Supplemental Table S3 continued

	L50AP31A2	**L61P8B12**	**L71BP33D1**	**L76P9E4**	**L106P3B10**
MA	0.349	0.205	0.001	0.013	0.184
PA	0.091	2.828	0.021	0.272	0.032
POA	0.040	4.667	0.039	0.270	0.016
SA	0.016	1.211	0.223	1.805	0.009
OA	0.013	4.015	0.392	4.231	0.015
LA	0.008	0.916	0.302	1.387	0.010
LNA	0.008	0.450	0.084	0.231	0.007
AA	0.005	0.201	2.085	0.866	0.006
DHA	0.001	0.084	1.733	0.878	0.004
	L118P4H5	**L119P3E5**	**L127P3G2**	**L138P1H8N24C**	**L156P3F926C**
MA	0.002	0.133	0.000	0.030	0.290
PA	0.017	0.423	0.003	0.141	2.078
POA	0.005	0.071	0.001	0.018	14.581
SA	0.018	0.171	0.014	0.112	4.779
OA	0.037	0.133	0.029	0.259	10.687
LA	0.018	0.068	0.020	0.124	3.141
LNA	0.008	0.035	0.005	0.461	1.435
AA	0.039	0.027	0.906	0.018	0.758
DHA	0.188	0.010	0.238	0.028	0.401

Supplemental Table S4. Error simulations for FFAu profiling as functions of the R value CV and the total FFAu concentration. The R value CVs range from 0% (for the "true" FFAu profile) to 5% and the total simulated FFAu concentrations were 0.5, 1.5 and 5.0 nM. The simulated profiles are in mole fractions for each of the 9 FFA of Table 1. The CV values for each FFAu were determined from the fits to 20 simulated FFAu profiles whose underlying R values were randomly altered by 1 to 2 % about the "true" profile R values (0%).

		Mole Fraction FFAu								
R value CV	**Total FFAu**	**MA**	**PA**	**POA**	**SA**	**OA**	**LA**	**LNA**	**AA**	**DHA**
0%	**CV**	0.22	0.135	0.137	0.022	0.335	0.038	0.042	0.059	0.013
1%	**0.496**	0.210	0.139	0.139	0.023	0.335	0.042	0.037	0.057	0.019
	3%	13%	19%	16%	39%	8%	41%	72%	13%	46%
	1.506	0.220	0.134	0.136	0.022	0.335	0.036	0.044	0.058	0.014
	1%	5%	7%	6%	15%	3%	20%	23%	5%	31%
	5.012	0.221	0.132	0.137	0.023	0.335	0.038	0.043	0.059	0.013
	1%	3%	5%	4%	12%	1%	9%	8%	2%	12%
2%	**0.484**	0.201	0.138	0.186	0.026	0.279	0.020	0.084	0.054	0.012
	13%	41%	66%	49%	71%	34%	109%	56%	31%	112%
	1.502	0.227	0.126	0.142	0.025	0.330	0.041	0.038	0.059	0.012
	3%	10%	13%	10%	20%	4%	35%	49%	14%	73%
	5.008	0.222	0.134	0.136	0.021	0.337	0.035	0.044	0.058	0.013
	1%	4%	7%	6%	24%	3%	21%	22%	4%	20%

Supplemental Table S5: HSA – FFA equilibrium dissociation constants (K_d). Measurements were performed at 22^0C. The standard deviations (SD) of at least two measurements are in nM. The BSA results were determined previously (10).

FFA	No. Sites	K_d (nM)	SD	BSA Sites	BSA K_d	BSA SD
MA	6	50.1	9.2			
PA	6	10.7	1.0	7	8.0	0.2
POA	5	26.8	0.2	7	38	2
SA	5	4.3	0.5	6	4.1	0.2
OA	7	4.7	0.6	7	5.9	0.1
LA	6	15.2	1	7	13	2
LNA	7	48.0	2.8	7	35	1
AA	8	11.7	1.2	7	16	1
DHA	7	22.2	1.3			