Association of Physical Activity with the Bioactive Lipidome and Incident Cardiovascular Events – Results from the VITamin D and OmegA-3 TriaL (VITAL) and JUPITER studies

Rosangela A. Hoshi, Yanyan Liu, Heike Luttmann-Gibson, Saumya Tiwari, Franco Giulianini, Allen M. Andres, Jeramie D. Watrous, Nancy R. Cook, Trisha Copeland, Karen H. Costenbader, Olivia I. Okereke, Paul M Ridker, JoAnn E. Manson, I-Min Lee, Vinayagamoorthy, Manickavasagar, Susan Cheng, Mohit Jain, Daniel I. Chasman, Olga V. Demler, Samia Mora

SUPPLEMENTAL MATERIAL

Table of contents

Supplemental Methods

Supplemental tables

Supplemental Figures

Supplemental Methods

Blood Sample Collection

VITAL-CTSC participants baseline visits took place between January 2012 and March 2014 in which they provided fasting blood samples. Visits to the CTSC provided the opportunity for face-to-face contact, allowing for detailed phenotyping and in-person validation of the remote assessment methods used in the main trial and ancillary studies. Both VITAL-CTSC and VITAL-CVD subsets, a baseline blood sample was provided voluntarily pre-main trial randomization. Participants were mailed a blood collection kit that contained a consent form, supplies and instructions for blood draw, and a cold pack and were returned via prepaid overnight courier. Upon arrival, samples were centrifuged and aliquoted into cryotubes as plasma, buffy coat, and red blood cells, and stored in liquid nitrogen freezers at a temperature of ≤ −130 ◦C. The process was completed within several hours of specimen receipt and the vast majority of samples were frozen within 30–36 h after venipuncture.

As part of the JUPITER protocol, all study participants provided a blood sample before randomization. Part of these blood samples were assayed in a core laboratory for LDL-C, HDL-C, apo B, and hsCRP as previously described [15,21], and other part were frozen and stored for future analyses.

Non-targeted Metabolomic Measurements with LC-MS Assay

Blood samples from both main studies were stored in vapor-phase liquid nitrogen (-170°C), thawed, separated into aliquots, refrozen, and shipped on dry ice to the University of California, San Diego. Approximately 11,000 features were extracted by using the method described in

Watrous et al. (2019). In addition to experimental and pooled plasma samples, a library of chemical standards (mostly from Cayman Chemicals) was run. Features from the experimental samples were then matched to those of the chemical standards library based on their m/z, retention times and fragmentation mass spectrum. Tandem plasma samples from respective case and control participants were randomly placed in neighboring wells on a plate to minimize effects of instrument drifts and were blindly assayed. Internal standards were inserted in each well and three pooled plasma samples were placed at the beginning in the middle and at the end of each plate to monitor for any drift in data quality. Annotations of BAL in datasets were verified further by examining the raw spectral data. Additionally, as BAL with high correlations can be features of the same compound, raw spectral data were used to resolve these instances to avoid the same compound appearing more than once in the dataset.

Eicosanoids and related oxylipins were measured through a directed non-targeted mass spectrometry approach, developed by Watrous et al. [17], that expanded the repertoire of oxylipins assayed in humans is and fully described elsewhere, using high mass accuracy LC-MS for measurement of bioactive lipid species. Briefly, using chemical networking of MS/MS spectral fragments and system analysis of chemical patterning, they found distinct oxylipin entities in human plasma, including compounds not previously documented in humans, and unknown compounds. Then, three databases, including an in-house database from oxylipin commercial standards were used for identification. Only the top scoring hit was considered for all matches to library reference spectra. All resulting matches were manually checked for consistency in fragmentation patterns between the library reference and experimental spectra. Finally, Custom Library Search for all known, putative known and putative novel oxylipins was performed using an RT tolerance of 0.1 minutes and a mass tolerance of ±5 ppm. Resulting features were manually denoised by visual inspection using the Mzmine peak list viewer where features exhibiting abnormal/poor peak shapes, inconsistent peak shapes and/or drastic shifts in retention time were deleted.

PA assessment

In VITAL, PA was assessed through a self-administrated questionnaire with questions regarding the average amount of time during the last 12 months spent in each of the following activities per week: jogging (i.e., > 10-minute miles), running (i.e., < 10-minute miles), aerobic exercise, aerobic dance, exercise machines, tennis, squash, racquetball, or lap swimming – defined as vigorous activities that require ≥ 6 metabolic equivalent of task (METs); walking or hiking (normal, brisk or very brisk pace, including walking to work), bicycling (including stationary bike), lower intensity exercise, yoga, stretching, toning, weight lifting, strength training, or other exercises (not considering stairs climbing) – defined as moderate activities that require 3 to 5.9 METs; and slow walking - defined as light activity that requires < 3METs; The total reported PA was calculated as weekly energy expenditure in METs by multiplying the intensity of each type of PA (MET) and time spent in the activity according to the questionnaire. Participants from VITAL with self-reported MET-hrs/wk ≥ 3SD from the mean were considered outliers and excluded from the analysis. PA continuous values were shifted and rescaled to mean 0 and SD = 1 for better comparison across studies and more interpretable results.

In JUPITER, PA was treated as an ordinal variable ranging from 1 to 6, according to the category of self-reported frequency per week: 1) Rarely/Never, 2) less than once a week, 3) once a week, 4) 2-3 times a week, 5) 4-6 times a week, or 6) daily.

Supplemental tables

Table S1. β coefficients and 95% confidence interval from model 2 (adjusted for age, sex, race/ethnicity, LDL-C, total-C, and smoking) of 145 BAL significantly associated with PA in VITAL-CTSC (FDR <.1) and validated in JUPITER-NC (FDR <.1)

BAL *m/z*	rt	VITAL-CTSC			JUPITER-NC			Annotation
		β coefficient	**95% CI**	**P-value***	**β coefficient**	**95% CI**	**P-value***	
225.113	1.87	0.09	0.03; 0.15	$4.0x10^{-03}$	0.06	0.01; 0.10	$1.5x10^{-02}$	--
228.16	2.53	0.16	0.10; 0.22	$3.9x10^{-07}$	0.08	0.04; 0.13	$1.6x10^{-04}$	--
236.6398	2.65	-0.08	-0.14; -0.02	$1.1x10^{-02}$	-0.08	-0.12; -0.04	$2.4x10^{-04}$	--
237.1493	3.96	0.09	0.02; 0.15	$7.2x10^{-03}$	0.07	0.03; 0.12	$1.6x10^{-03}$	--
237.1494	4.48	0.12	0.06; 0.18	$1.9x10^{-04}$	0.06	0.02; 0.11	$7.6x10^{-03}$	--
237.1495	3.23	0.13	0.07; 0.20	$2.6x10^{-05}$	0.09	0.05; 0.13	$6.2x10^{-05}$	--
237.1495	4.42	0.12	0.06; 0.18	$1.9x10^{-04}$	0.08	0.03; 0.12	$9.3x10^{-04}$	--
237.1859	5.46	0.13	0.07; 0.19	$2.6x10^{-05}$	0.07	0.02; 0.11	$2.4x10^{-03}$	--
239.1287	1.84	0.11	0.05; 0.17	$6.6x10^{-04}$	0.06	0.02; 0.11	$5.4x10^{-03}$	--
241.1808	3.27	-0.1	-0.15; -0.04	$8.0x10^{-04}$	-0.07	-0.11; -0.02	$1.8x10^{-03}$	--
243.1965	5.99	0.1	0.04; 0.16	$1.2x10^{-03}$	0.07	0.03; 0.12	$8.9x10^{-04}$	--
245.0863	2.79	0.09	0.03; 0.15	$5.4x10^{-03}$	0.09	0.04; 0.13	$1.4x10^{-04}$	--
251.1289	2.63	0.1	0.03; 0.16	$2.9x10^{-03}$	0.06	0.02; 0.11	$8.1x10^{-03}$	--
251.166	4.83	0.15	0.09; 0.21	$2.6x10^{-06}$	0.08	0.03; 0.12	$8.4x10^{-04}$	--
251.2015	6.38	-0.12	-0.18; -0.06	$4.9x10^{-05}$	-0.05	-0.09; -0.01	$1.8x10^{-02}$	--
251.2017	6.31	-0.14	-0.20; -0.09	$4.3x10^{-07}$	-0.05	-0.09; -0.01	$1.2x10^{-02}$	--
253.1445	2.71	0.09	0.03; 0.15	$4.9x10^{-03}$	0.07	0.02; 0.11	$4.4x10^{-03}$	--
253.1446	2.34	0.09	0.03; 0.15	$5.6x10^{-03}$	0.07	0.02; 0.11	$4.5x10^{-03}$	--
253.1447	1.95	0.13	0.07; 0.19	$4.6x10^{-05}$	0.06	0.01; 0.11	$9.5x10^{-03}$	--
253.1448	2.65	0.12	0.06; 0.18	$1.7x10^{-04}$	0.07	0.03; 0.12	$1.4x10^{-03}$	--
254.2226	6.51	-0.08	-0.14; -0.03	$4.4x10^{-03}$	-0.05	-0.10; -0.01	$1.3x10^{-02}$	--
257.1759	2.86	0.17	0.11; 0.23	$7.3x10^{-08}$	0.07	0.03; 0.12	$1.4x10^{-03}$	--

257.1761	2.81	0.17	0.11; 0.23	7.3x10^{-08}	0.07	0.03; 0.12	2.1x10^{-03}	--
257.1762	2.89	0.17	0.11; 0.23	7.3x10^{-08}	0.1	0.06; 0.15	5.2x10^{-06}	--
265.1813	5.28	0.16	0.10; 0.22	4.3x10^{-07}	0.09	0.05; 0.14	8.5x10^{-05}	--
267.1597	2.3	0.1	0.03; 0.16	2.4x10^{-03}	0.07	0.02; 0.11	4.1x10^{-03}	--
270.0751	1.53	0.13	0.07; 0.19	5.6x10^{-05}	0.06	0.02; 0.11	6.9x10^{-03}	--
283.1531	2.77	0.16	0.10; 0.22	6.3x10^{-07}	0.06	0.02; 0.11	5.4x10^{-03}	--
285.1711	2.66	0.12	0.06; 0.18	1.2x10^{-04}	0.06	0.01; 0.10	1.4x10^{-02}	--
287.2231	6.08	0.12	0.06; 0.19	5.6x10^{-05}	0.07	0.03; 0.12	1.8x10^{-03}	--
289.1475	3.94	0.11	0.06; 0.17	1.7x10^{-04}	0.06	0.01; 0.10	1.1x10^{-02}	--
291.1241	2.09	0.08	0.02; 0.15	8.8x10^{-03}	0.05	0.01; 0.10	1.8x10^{-02}	--
295.153	3.32	0.13	0.07; 0.20	2.4x10^{-05}	0.06	0.01; 0.10	1.5x10^{-02}	--
295.1531	3.24	0.08	0.02; 0.14	1.4x10^{-02}	0.08	0.03; 0.12	7.5x10^{-04}	--
295.189	3.32	0.08	0.02; 0.14	1.1x10^{-02}	0.06	0.01; 0.10	1.1x10^{-02}	--
297.1702	4.42	0.12	0.06; 0.18	1.4x10^{-04}	0.07	0.03; 0.12	2.3x10^{-03}	--
300.2004	2.73	0.1	0.04; 0.16	1.4x10^{-03}	0.06	0.01; 0.10	1.5x10^{-02}	--
307.0292	1.81	0.18	0.12; 0.24	7.6x10^{-09}	0.09	0.05; 0.13	7.0x10^{-05}	--
307.0317	1.7	0.18	0.12; 0.24	7.6x10^{-09}	0.07	0.03; 0.12	2.0x10^{-03}	--
307.1199	2.04	0.1	0.04; 0.16	8.0x10^{-04}	0.08	0.04; 0.12	3.1x10^{-04}	--
307.2281	6.28	0.14	0.08; 0.21	5.0x10^{-06}	0.07	0.02; 0.11	3.4x10^{-03}	--
311.2231	3.02	0.16	0.10; 0.22	4.7x10^{-07}	0.06	0.02; 0.11	5.0x10^{-03}	--
313.1488	3.96	0.13	0.07; 0.19	2.0x10^{-05}	0.07	0.02; 0.11	2.4x10^{-03}	--
313.2387	3.46	0.14	0.08; 0.20	3.0x10^{-06}	0.07	0.02; 0.11	2.3x10^{-03}	12,13-diHOME
313.2387	3.59	0.13	0.06; 0.19	5.9x10^{-05}	0.06	0.01; 0.10	9.6x10^{-03}	9,10-diHOME
314.1013	2	0.12	0.06; 0.18	2.3x10^{-04}	0.06	0.01; 0.10	1.1x10^{-02}	--
315.164	4.68	0.16	0.10; 0.22	3.4x10^{-07}	0.06	0.01; 0.10	1.4x10^{-02}	--
317.2255	6.72	-0.09	-0.16; -0.03	3.0x10^{-03}	-0.06	-0.11; -0.02	5.9x10^{-03}	--
318.1743	2.1	-0.1	-0.17; -0.04	7.6x10^{-04}	-0.08	-0.12; -0.03	5.6x10^{-04}	--
319.1542	4.41	0.11	0.05; 0.17	5.4x10^{-04}	0.07	0.03; 0.12	1.1x10^{-03}	--
320.1953	3.61	0.08	0.02; 0.15	7.5x10^{-03}	0.08	0.03; 0.12	1.0x10^{-03}	--
323.1864	4.44	0.1	0.04; 0.16	1.9x10^{-03}	0.07	0.03; 0.12	1.8x10^{-03}	--

323.2234	4.08	0.13	0.07; 0.19	$2.1x10^{-05}$	0.07	0.03; 0.12	$1.3x10^{-03}$	--
325.2726	5.72	0.1	0.04; 0.16	$1.2x10^{-03}$	0.08	0.03; 0.12	$5.0x10^{-04}$	--
327.2907	6.06	0.1	0.04; 0.16	$1.2x10^{-03}$	0.06	0.02; 0.11	$7.6x10^{-03}$	--
333.1398	4.42	0.1	0.04; 0.17	$1.2x10^{-03}$	0.06	0.02; 0.11	$8.1x10^{-03}$	--
341.2703	3.72	0.22	0.16; 0.29	$1.6x10^{-12}$	0.13	0.08; 0.17	$1.2x10^{-08}$	--
343.2856	4.3	0.23	0.17; 0.30	$8.0x10^{-14}$	0.11	0.06; 0.15	$1.9x10^{-06}$	--
343.2858	3.97	0.09	0.02; 0.15	$7.1x10^{-03}$	0.06	0.02; 0.11	$6.9x10^{-03}$	--
346.1791	1.85	0.12	0.05; 0.18	$2.1x10^{-04}$	0.06	0.01; 0.10	$1.4x10^{-02}$	--
359.28	3.32	0.15	0.09; 0.21	$2.3x10^{-06}$	0.06	0.02; 0.11	$3.6x10^{-03}$	--
387.2758	6.2	-0.13	-0.19; -0.07	$1.4x10^{-05}$	-0.06	-0.10; -0.02	$6.9x10^{-03}$	--
393.2679	2.31	-0.1	-0.16; -0.04	$9.5x10^{-04}$	-0.07	-0.11; -0.02	$4.5x10^{-03}$	--
398.213	4.95	0.1	0.04; 0.16	$1.3x10^{-03}$	0.08	0.03; 0.12	$5.6x10^{-04}$	--
411.1939	1.98	-0.08	-0.14; -0.02	$5.8x10^{-03}$	-0.05	-0.09; -0.01	$1.8x10^{-02}$	--
413.2009	1.67	-0.09	-0.15; -0.03	$4.0x10^{-03}$	-0.06	-0.10; -0.01	$9.7x10^{-03}$	--
415.3093	6.5	-0.12	-0.18; -0.06	$1.6x10^{-04}$	-0.05	-0.10; -0.01	$1.8x10^{-02}$	Oleoyl-glycerol
415.3229	6.36	0.1	0.04; 0.16	$1.7x10^{-03}$	0.06	0.02; 0.11	$4.7x10^{-03}$	--
417.2658	3.69	-0.09	-0.16; -0.03	$2.6x10^{-03}$	-0.08	-0.13; -0.04	$1.6x10^{-04}$	--
417.3002	6.43	-0.08	-0.14; -0.02	$7.6x10^{-03}$	-0.05	-0.10; -0.01	$1.6x10^{-02}$	--
419.2079	1.4	0.1	0.04; 0.17	$9.2x10^{-04}$	0.06	0.02; 0.10	$7.4x10^{-03}$	Cortisone
429.2088	1.56	-0.09	-0.15; -0.03	$3.5x10^{-03}$	-0.07	-0.12; -0.03	$1.3x10^{-03}$	--
431.317	4.61	-0.08	-0.14; -0.02	$9.2x10^{-03}$	-0.06	-0.10; -0.02	$5.7x10^{-03}$	--
443.1562	2.2	0.11	0.04; 0.17	$1.0x10^{-03}$	0.05	0.01; 0.10	$1.7x10^{-02}$	--
443.1562	2.29	0.08	0.02; 0.14	$1.2x10^{-02}$	0.06	0.01; 0.10	$9.7x10^{-03}$	--
444.1599	2.22	0.11	0.05; 0.17	$4.9x10^{-04}$	0.06	0.02; 0.10	$8.8x10^{-03}$	--
445.2957	3.92	-0.11	-0.18; -0.05	$2.6x10^{-04}$	-0.09	-0.13; -0.04	$7.8x10^{-05}$	--
446.2902	2.73	-0.09	-0.15; -0.03	$5.6x10^{-03}$	-0.06	-0.10; -0.01	$1.4x10^{-02}$	--
446.3008	3.91	-0.12	-0.18; -0.06	$7.8x10^{-05}$	-0.08	-0.12; -0.03	$6.8x10^{-04}$	--
447.3121	6.28	0.13	0.07; 0.19	$3.6x10^{-05}$	0.08	0.03; 0.12	$1.0x10^{-03}$	--
449.2906	2.65	-0.09	-0.16; -0.03	$3.5x10^{-03}$	-0.06	-0.11; -0.02	$7.9x10^{-03}$	--
457.2808	3.57	-0.09	-0.16; -0.03	$2.5x10^{-03}$	-0.06	-0.10; -0.02	$8.4x10^{-03}$	--

457.3313	5.26	0.09	0.02; 0.15	$6.8x10^{-03}$	0.06	0.01; 0.10	$1.3x10^{-02}$	--
462.2869	2.08	-0.08	-0.14; -0.02	$7.5x10^{-03}$	-0.06	-0.10; -0.01	$1.6x10^{-02}$	--
474.2872	2.65	-0.11	-0.17; -0.05	$7.2x10^{-04}$	-0.07	-0.11; -0.03	$1.2x10^{-03}$	--
475.343	5.68	0.12	0.06; 0.18	$9.8x10^{-05}$	0.08	0.04; 0.13	$2.4x10^{-04}$	--
475.3434	4.76	-0.09	-0.15; -0.02	$6.5x10^{-03}$	-0.05	-0.10; -0.01	$1.7x10^{-02}$	--
476.156	1.36	0.09	0.02; 0.15	$6.6x10^{-03}$	0.06	0.02; 0.11	$6.8x10^{-03}$	Oxymorphone 3b-D-glucuronide
479.3403	3.94	-0.13	-0.19; -0.07	$2.8x10^{-05}$	-0.06	-0.10; -0.01	$1.1x10^{-02}$	--
489.197	1.91	0.09	0.03; 0.15	$4.9x10^{-03}$	0.06	0.02; 0.11	$6.7x10^{-03}$	--
493.3492	4.4	0.09	0.03; 0.16	$3.8x10^{-03}$	0.06	0.02; 0.10	$4.8x10^{-03}$	--
497.2762	2.12	0.14	0.07; 0.20	$1.9x10^{-05}$	0.07	0.02; 0.11	$4.0x10^{-03}$	--
497.2791	1.36	0.15	0.09; 0.22	$1.8x10^{-06}$	0.06	0.02; 0.11	$5.4x10^{-03}$	--
497.3495	2.7	-0.14	-0.20; -0.08	$6.9x10^{-06}$	-0.06	-0.10; -0.01	$1.3x10^{-02}$	--
503.3233	3.35	0.09	0.03; 0.15	$4.3x10^{-03}$	0.05	0.01; 0.09	$1.7x10^{-02}$	--
504.3162	4.6	0.13	0.07; 0.19	$2.7x10^{-05}$	0.07	0.02; 0.11	$3.4x10^{-03}$	LysoPC(15:0)
507.3339	3.08	0.15	0.09; 0.21	$3.7x10^{-06}$	0.07	0.03; 0.12	$1.5x10^{-03}$	--
508.308	2.64	-0.16	-0.22; -0.10	$1.4x10^{-07}$	-0.08	-0.12; -0.04	$2.5x10^{-04}$	--
509.2371	2.24	0.1	0.04; 0.17	$1.0x10^{-03}$	0.06	0.01; 0.10	$1.5x10^{-02}$	--
509.2386	2.2	0.1	0.04; 0.17	$1.0x10^{-03}$	0.06	0.02; 0.11	$7.4x10^{-03}$	--
511.4187	6.84	0.09	0.03; 0.15	$4.0x10^{-03}$	0.06	0.01; 0.10	$1.4x10^{-02}$	--
515.2827	1.91	0.08	0.02; 0.15	$7.2x10^{-03}$	0.06	0.01; 0.10	$1.3x10^{-02}$	--
515.3282	3.69	0.11	0.05; 0.17	$4.4x10^{-04}$	0.08	0.03; 0.12	$9.6x10^{-04}$	--
519.3196	2.66	0.14	0.08; 0.20	$8.1x10^{-06}$	0.06	0.02; 0.10	$7.8x10^{-03}$	--
525.2711	1.87	-0.1	-0.16; -0.03	$2.3x10^{-03}$	-0.09	-0.13; -0.05	$5.6x10^{-05}$	--
532.2527	4.17	-0.12	-0.18; -0.06	$1.3x10^{-04}$	-0.08	-0.12; -0.04	$1.1x10^{-04}$	--
533.334	2.92	0.11	0.05; 0.18	$3.9x10^{-04}$	0.06	0.02; 0.11	$5.2x10^{-03}$	--
537.329	1.91	0.08	0.02; 0.14	$9.1x10^{-03}$	0.09	0.04; 0.13	$1.2x10^{-04}$	--
537.3295	2.01	0.1	0.05; 0.16	$4.8x10^{-04}$	0.05	0.01; 0.09	$9.0x10^{-03}$	--
541.2657	1.4	-0.12	-0.18; -0.06	$6.1x10^{-05}$	-0.06	-0.11; -0.02	$6.5x10^{-03}$	--
543.2787	1.24	-0.16	-0.22; -0.10	$3.1x10^{-07}$	-0.07	-0.11; -0.02	$3.7x10^{-03}$	--

553.4885	6.67	-0.1	-0.16; -0.04	$1.3x10^{-03}$	-0.06	-0.11; -0.02	$7.0x10^{-03}$	--
555.3118	4.59	0.15	0.09; 0.22	$8.7x10^{-07}$	0.1	0.05; 0.14	$1.3x10^{-05}$	--
557.3039	4.59	0.18	0.12; 0.24	$8.1x10^{-09}$	0.07	0.03; 0.12	$1.1x10^{-03}$	--
564.3362	4.81	0.12	0.06; 0.18	$1.4x10^{-04}$	0.05	0.01; 0.10	$1.8x10^{-02}$	--
564.368	4.26	-0.13	-0.19; -0.07	$2.1x10^{-05}$	-0.06	-0.10; -0.01	$1.0x10^{-02}$	--
575.3607	4.63	0.1	0.04; 0.16	$1.9x10^{-03}$	0.08	0.04; 0.13	$3.5x10^{-04}$	--
577.3763	4.53	-0.12	-0.18; -0.06	$8.6x10^{-05}$	-0.06	-0.10; -0.01	$1.3x10^{-02}$	--
578.3502	4.57	0.14	0.08; 0.20	$2.2x10^{-06}$	0.08	0.03; 0.12	$4.6x10^{-04}$	--
582.271	4.66	-0.11	-0.18; -0.05	$2.1x10^{-04}$	-0.06	-0.11; -0.02	$4.6x10^{-03}$	--
582.3783	6.2	0.08	0.02; 0.14	$1.4x10^{-02}$	0.06	0.01; 0.10	$1.6x10^{-02}$	--
594.3428	2.42	0.12	0.05; 0.18	$2.7x10^{-04}$	0.06	0.02; 0.10	$8.4x10^{-03}$	--
594.3488	2.48	0.1	0.04; 0.16	$1.6x10^{-03}$	0.07	0.02; 0.11	$2.6x10^{-03}$	--
598.3736	3.26	0.09	0.03; 0.15	$3.0x10^{-03}$	0.06	0.01; 0.10	$1.3x10^{-02}$	--
598.3744	3.19	0.09	0.03; 0.15	$3.0x10^{-03}$	0.06	0.01; 0.10	$8.9x10^{-03}$	--
599.3444	1.83	-0.12	-0.18; -0.06	$1.1x10^{-04}$	-0.06	-0.10; -0.01	$1.3x10^{-02}$	--
605.4063	6.74	0.08	0.02; 0.14	$5.7x10^{-03}$	0.09	0.05; 0.14	$1.5x10^{-05}$	--
611.3527	2.01	-0.08	-0.14; -0.02	$1.4x10^{-02}$	-0.07	-0.12; -0.03	$1.2x10^{-03}$	--
611.3807	3.45	-0.12	-0.18; -0.06	$1.4x10^{-04}$	-0.06	-0.10; -0.01	$1.3x10^{-02}$	--
611.3808	3.37	-0.12	-0.18; -0.06	$1.9x10^{-04}$	-0.07	-0.12; -0.03	$8.0x10^{-04}$	--
611.3809	3.18	-0.1	-0.16; -0.04	$1.3x10^{-03}$	-0.07	-0.11; -0.03	$1.3x10^{-03}$	--
611.3813	2.97	-0.12	-0.18; -0.06	$1.1x10^{-04}$	-0.08	-0.12; -0.03	$7.8x10^{-04}$	--
613.3591	2.06	-0.1	-0.16; -0.04	$1.5x10^{-03}$	-0.06	-0.10; -0.01	$1.1x10^{-02}$	--
613.3866	2.95	-0.13	-0.19; -0.07	$2.5x10^{-05}$	-0.06	-0.11; -0.02	$4.0x10^{-03}$	--
616.2999	4.59	0.12	0.06; 0.18	$7.9x10^{-05}$	0.07	0.03; 0.12	$1.3x10^{-03}$	--
621.3285	3.58	0.08	0.02; 0.15	$6.8x10^{-03}$	0.06	0.01; 0.10	$9.0x10^{-03}$	--
623.2711	1.37	-0.15	-0.21; -0.08	$2.9x10^{-06}$	-0.07	-0.12; -0.03	$1.4x10^{-03}$	--
625.3549	2.41	-0.09	-0.15; -0.03	$2.1x10^{-03}$	-0.07	-0.11; -0.03	$1.1x10^{-03}$	--
626.3601	2.41	-0.1	-0.16; -0.04	$1.5x10^{-03}$	-0.06	-0.10; -0.02	$5.3x10^{-03}$	--
627.3758	2.45	-0.08	-0.14; -0.02	$9.8x10^{-03}$	-0.07	-0.11; -0.02	$3.8x10^{-03}$	--
634.2473	4.1	0.09	0.03; 0.15	$5.2x10^{-03}$	0.07	0.02; 0.11	$4.1x10^{-03}$	--

635.38	6.35	0.15	0.08; 0.21	$4.0x10^{-06}$	0.1	0.06; 0.15	$9.0x10^{-06}$	--
635.5287	6.49	-0.1	-0.16; -0.04	$1.8x10^{-03}$	-0.06	-0.11; -0.02	$3.7x10^{-03}$	--
636.2644	4.59	0.11	0.05; 0.17	$6.7x10^{-04}$	0.08	0.04; 0.12	$3.7x10^{-04}$	--
648.3896	6.06	0.17	0.11; 0.23	$3.3x10^{-08}$	0.06	0.02; 0.11	$4.5x10^{-03}$	--

BAL – Bioactive lipid; m/z – mass to charge ratio; rt – retention time. *Unadjusted P-value

Table S2. 95% Confidence Intervals from original linear regression model and after bootstrapped replication for BALs which residuals were non-normally distributed and measured values failed to be significantly different in high PA levels *vs*. low PA.

Study	Bioactive Lipid (*m/z* rt)	Original 95%CI	Bootstrapped 95%CI
VITAL-CTSC	295.1528 3.17	0.016; 0.141	0.014; 0.137
	237.1494 3.90	0.024; 0.149	0.026; 0.154
	253.1441 2.69	0.027; 0.153	0.024; 0.153
JUPITER-NC	359.2800 3.32	0.020; 0.101	0.021; 0.105
	457.3313 5.26	0.012; 0.099	0.011; 0.098
	225.1130 1.87	0.010; 0.097	0.012; 0.093

Table S3. β coefficients and 95% confidence interval from model 3 (adjusted for age, sex, race/ethnicity, LDL-C, total-C, smoking, BMI categories, and HDL) of 45 BAL significantly associated with PA in VITAL-CTSC (FDR <.1) and validated in JUPITER-NC (nominal P < .05)

BAL *m/z*	rt	VITAL-CTSC			JUPITER-NC			Annotation
		β coefficient	95% CI	P-value*	β coefficient	95% CI	P-value*	
228.1600	2.53	0.12	0.06; 0.19	$1.1x10^{-04}$	0.08	0.04; 0.12	$1.9x10^{-04}$	--
237.1495	3.23	0.1	0.04; 0.16	$1.9x10^{-03}$	0.09	0.04; 0.13	$1.0x10^{-04}$	--
241.1808	4.64	0.11	0.05; 0.18	$3.8x10^{-04}$	0.05	0.00; 0.09	$3.3x10^{-02}$	--
257.1759	2.86	0.11	0.05; 0.17	$4.6x10^{-04}$	0.06	0.01; 0.10	$1.3x10^{-02}$	--
257.1761	2.81	0.11	0.05; 0.17	$4.6x10^{-04}$	0.06	0.02; 0.10	$7.3x10^{-03}$	--
257.1762	2.89	0.11	0.05; 0.17	$4.6x10^{-04}$	0.09	0.05; 0.13	$4.3x10^{-05}$	--
265.1813	5.28	0.1	0.04; 0.17	$1.1x10^{-03}$	0.08	0.04; 0.12	$3.6x10^{-04}$	--
267.1241	3.06	0.11	0.05; 0.18	$6.3x10^{-04}$	0.05	0.01; 0.09	$9.6x10^{-03}$	--
285.1711	2.66	0.1	0.04; 0.16	$1.7x10^{-03}$	0.07	0.02; 0.11	$2.6x10^{-03}$	--
287.2231	6.08	0.11	0.05; 0.18	$3.4x10^{-04}$	0.07	0.03; 0.11	$2.1x10^{-03}$	--
289.1475	3.94	0.11	0.05; 0.17	$3.7x10^{-04}$	0.06	0.02; 0.10	$5.2x10^{-03}$	--
307.0292	1.81	0.17	0.10; 0.23	$2.6x10^{-07}$	0.08	0.04; 0.12	$3.1x10^{-04}$	--
307.0317	1.7	0.17	0.10; 0.23	$2.6x10^{-07}$	0.06	0.02; 0.11	$3.8x10^{-03}$	--
307.2281	6.28	0.13	0.07; 0.20	$3.4x10^{-05}$	0.07	0.03; 0.11	$1.7x10^{-03}$	--
311.2231	3.02	0.13	0.06; 0.19	$8.9x10^{-05}$	0.05	0.01; 0.10	$1.2x10^{-02}$	--
313.1488	3.96	0.11	0.05; 0.18	$3.0x10^{-04}$	0.07	0.03; 0.11	$1.2x10^{-03}$	--
313.2387	3.46	0.11	0.05; 0.17	$3.4x10^{-04}$	0.07	0.02; 0.11	$3.4x10^{-03}$	12,13-diHOME
315.1640	4.68	0.13	0.07; 0.20	$2.1x10^{-05}$	0.07	0.02; 0.11	$2.6x10^{-03}$	--
317.2255	6.72	-0.11	-0.17; -0.04	$1.0x10^{-03}$	-0.05	-0.09; -0.01	$1.0x10^{-02}$	--
323.2234	4.08	0.12	0.06; 0.18	$2.2x10^{-04}$	0.07	0.02; 0.11	$3.4x10^{-03}$	--
341.2703	3.72	0.11	0.05; 0.16	$1.8x10^{-04}$	0.1	0.06; 0.14	$4.9x10^{-06}$	--
343.2491	4.25	0.13	0.07; 0.20	$2.9x10^{-05}$	0.05	0.01; 0.09	$1.6x10^{-02}$	--
343.2856	4.3	0.12	0.06; 0.18	$2.7x10^{-05}$	0.08	0.04; 0.12	$2.4x10^{-04}$	--
349.1273	3.06	0.1	0.04; 0.17	$1.3x10^{-03}$	0.05	0.01; 0.09	$7.0x10^{-03}$	--

365.0946	3.06	0.11	0.05; 0.17	$7.5x10^{-04}$	0.05	0.01; 0.09	$1.6x10^{-02}$	--
398.2130	4.95	0.11	0.04; 0.17	$8.1x10^{-04}$	0.07	0.03; 0.11	$7.4x10^{-04}$	--
425.3365	6.44	0.11	0.04; 0.17	$1.1x10^{-03}$	0.05	0.00; 0.09	$3.1x10^{-02}$	--
441.3334	3.48	0.11	0.05; 0.17	$7.0x10^{-04}$	0.05	0.01; 0.10	$1.1x10^{-02}$	--
443.1562	2.2	0.12	0.05; 0.18	$4.2x10^{-04}$	0.06	0.02; 0.10	$3.6x10^{-03}$	--
443.1562	2.29	0.11	0.04; 0.17	$1.2x10^{-03}$	0.06	0.02; 0.11	$2.1x10^{-03}$	--
444.1599	2.22	0.12	0.06; 0.19	$2.5x10^{-04}$	0.06	0.02; 0.10	$2.6x10^{-03}$	--
447.3121	6.28	0.12	0.06; 0.18	$2.2x10^{-04}$	0.08	0.03; 0.12	$5.5x10^{-04}$	--
463.2344	1.63	-0.09	-0.15; -0.04	$1.9x10^{-03}$	-0.03	-0.07; 0.00	$4.2x10^{-02}$	--
469.2835	2.99	0.11	0.04; 0.17	$1.0x10^{-03}$	0.05	0.01; 0.10	$1.5x10^{-02}$	--
497.2762	2.12	0.13	0.07; 0.19	$6.6x10^{-05}$	0.06	0.02; 0.11	$6.6x10^{-03}$	--
497.2762	1.85	0.1	0.04; 0.17	$1.6x10^{-03}$	0.05	0.01; 0.10	$2.1x10^{-02}$	--
497.2768	2.07	0.13	0.07; 0.19	$6.6x10^{-05}$	0.04	0.00; 0.08	$4.5x10^{-02}$	--
497.2791	1.36	0.12	0.06; 0.19	$1.8x10^{-04}$	0.05	0.01; 0.09	$2.2x10^{-02}$	--
515.3282	3.69	0.11	0.04; 0.17	$1.4x10^{-03}$	0.07	0.02; 0.11	$3.0x10^{-03}$	--
533.3340	2.92	0.11	0.04; 0.17	$1.3x10^{-03}$	0.06	0.01; 0.10	$9.8x10^{-03}$	--
557.2396	3.06	0.11	0.04; 0.17	$8.3x10^{-04}$	0.05	0.01; 0.09	$1.8x10^{-02}$	--
557.3039	4.59	0.1	0.04; 0.16	$6.5x10^{-04}$	0.04	0.01; 0.07	$5.2x10^{-03}$	--
575.3607	4.63	0.1	0.03; 0.16	$2.8x10^{-03}$	0.07	0.03; 0.11	$8.0x10^{-04}$	--
582.2710	4.66	-0.1	-0.16; -0.04	$1.3x10^{-03}$	-0.05	-0.08; -0.01	$7.5x10^{-03}$	--
635.3800	6.35	0.12	0.06; 0.18	$2.6x10^{-04}$	0.09	0.05; 0.13	$4.5x10^{-05}$	--

BAL – Bioactive lipid; m/z – mass to charge ratio; rt – retention time. *Unadjusted P-value

Table S4. Two-sample Mendelian randomization results for associations between each BAL-SNPs combination and CHD

BAL *m/z* rt	β-coefficient	P-value*	N of variants	Variants included
613.3866 2.95	-0.07	$2.4x10^{-04}$	2	†rs11045856, †rs12367888
613.3591 2.06	-0.07	$3.3x10^{-04}$	2	†rs11045856, †rs12367888
611.3807 3.45	-0.05	$4.8x10^{-04}$	2	†rs11045856, †rs12367888
611.3813 2.97	-0.05	$7.5x10^{-04}$	3	†rs11045856, †rs12367888, rs78338680
611.3809 3.18	-0.05	$1.0x10^{-03}$	3	†rs11045856, †rs12367888, rs78338680
457.2808 3.57	-0.12	$3.4x10^{-03}$	2	†rs11045856, †rs12367888
508.3080 2.64	-0.06	$1.4x10^{-02}$	1	rs4997684
323.2234 4.08	-0.06	$2.1x10^{-02}$	1	†rs12367888
236.6398 2.65	-0.07	$2.2x10^{-02}$	1	†rs12367888
474.2872 2.65	-0.07	$2.2x10^{-02}$	1	†rs12367888
320.1953 3.61	-0.07	$2.3x10^{-02}$	1	†rs12367888
314.1013 2.00	-0.08	$3.0x10^{-02}$	1	rs16918645
582.2710 4.66	0.06	$5.4x10^{-02}$	1	†rs174592
318.1743 2.10	-0.09	$6.5x10^{-02}$	1	rs2035742
577.3763 4.53	-0.04	$7.3x10^{-02}$	1	rs2277119
285.1711 2.66	-0.03	$1.2x10^{-01}$	2	†rs5751777, rs79966373
497.2762 2.12	-0.02	$1.4x10^{-01}$	2	†rs10238028, †rs28468623
429.2088 1.56	0.02	$1.5x10^{-01}$	3	†rs10238028, †rs28468623, rs6705251
411.1939 1.98	0.04	$1.7x10^{-01}$	1	†rs6889699
598.3736 3.26	0.04	$1.9x10^{-01}$	1	rs111906477
598.3744 3.19	0.04	$1.9x10^{-01}$	1	rs111906477
594.3488 2.48	0.04	$1.9x10^{-01}$	1	rs111906477
594.3428 2.42	0.05	$1.9x10^{-01}$	1	rs111906477
537.3295 2.01	-0.03	$2.2x10^{-01}$	1	†rs34367065

503.3233 3.35	-0.04	$2.2x10^{-01}$	1	†rs34367065
283.1531 2.77	0.03	$3.3x10^{-01}$	1	†rs9943251
431.3170 4.61	-0.01	$4.8x10^{-01}$	1	†rs4663971
307.1199 2.04	-0.01	$5.5x10^{-01}$	1	†rs58231493
611.3527 2.01	0.01	$6.5x10^{-01}$	1	†rs10006452
228.1600 2.53	-0.01	$7.0x10^{-01}$	1	†rs11211408
245.0863 2.79	0.00	$9.8x10^{-01}$	2	rs187758672, †rs58231493

N of variants: number of variants associated with each BAL; †pleiotropic instruments according to Table S4. * Unadjusted P-value. BAL – Bioactive lipid; m/z – mass to charge ratio; rt – retention time.

Mendelian randomization performed with the R package "MendelianRandomization" (available at https://CRAN.R-project.org/package=MendelianRandomization).

Table S5. Pleiotropy table for previously known trait associations with index SNPs reported in the GWAS catalog [1]

Index SNP	Phenotype	Possible other SNPs	P-value	PMID
rs10006452	Estradiol levels	rs7662029	$4.00x10^{-18}$	34255042
rs10006452	Gamma glutamyl transferase levels	rs4588522	$5.00x10^{-09}$	33339817
rs10006452	Liver enzyme levels (alkaline phosphatase)	rs11931182	$3.00x10^{-12}$	33972514
rs10006452	Serum 25-Hydroxyvitamin D levels	rs6422323	$5.00x10^{-51}$	32242144
rs10006452	Systolic blood pressure	rs6422324	$1.00x10^{-08}$	30595370
rs10006452	Total testosterone levels	rs4632729	$2.00x10^{-30}$	32042192
rs10006452	Urinary metabolite levels in chronic kidney disease	rs7666195	$2.00x10^{-14}$	31959995
rs10238028	Lymphocyte counts	rs6465750	$5.00x10^{-23}$	32888494
rs10238028	Metabolite levels	rs10242455	$1.00x10^{-45}$	23093944
rs10238028	Sex hormone-binding globulin levels	rs10238028	$1.00x10^{-11}$	32042192
rs10238028	Tacrolimus trough concentration in kidney transplant patients	rs776746	$4.00x10^{-97}$	30801552
rs10238028	Urinary metabolite modules (eigenmetabolites) in chronic kidney disease	rs776746	$3.00x10^{-18}$	31959995
rs11045856	Serum metabolite levels	rs11045856	$6.00x10^{-40}$	33031748
rs11211408	Metabolite levels	rs6678639	$8.00x10^{-86}$	24816252
rs11211408	Serum metabolite concentrations in chronic kidney disease	rs4507958	$4.00x10^{-14}$	33838163
rs11211408	Metabolic traits	rs9332998	$5.00x10^{-32}$	21886157
rs12367888	AR-C124910XX levels in individuals with acute coronary syndromes treated with ticagrelor	rs113681054	$4.00x10^{-13}$	25935875

rs12367888	Bilirubin levels	rs4149056	7.00×10^{-13}	19414484
rs12367888	Bioavailable testosterone levels	rs4149056	3.00×10^{-35}	32042192
rs12367888	Clinical laboratory measurements	rs4149081	4.00×10^{-22}	27897004
rs12367888	Cystatin C levels	rs4149081	1.00×10^{-12}	33462484
rs12367888	Direct bilirubin levels	rs4149081	$0.00 \times 10^{+00}$	33462484
rs12367888	Fasting plasma glycochenodeoxycholate 3-O-glucuronide concentration	rs4149056	3.00×10^{-30}	32961594
rs12367888	Free thyroxine concentration	rs4149056	6.00×10^{-11}	30367059
rs12367888	Heel bone mineral density	rs4149056	4.00×10^{-12}	30595370
rs12367888	Low density lipoprotein cholesterol levels (on statin treatment)	rs58310495	2.00×10^{-11}	31969989
rs12367888	Low testosterone levels	rs4149056	7.00×10^{-16}	34337532
rs12367888	Lysophosphatidylethanolamine levels	rs1871395	4.00×10^{-06}	31551469
rs12367888	Mean corpuscular hemoglobin	rs4149056	9.00×10^{-25}	32888493
rs12367888	Mean corpuscular volume	rs4149056	6.00×10^{-25}	32888493
rs12367888	Mean spheric corpuscular volume	rs4149056	1.00×10^{-23}	32888494
rs12367888	Metabolic traits	rs4149081	3.00×10^{-22}	21886157
rs12367888	Metabolite levels	rs4149056	6.0×10^{-315}	24816252
rs12367888	Methotrexate clearance (acute lymphoblastic leukemia)	rs4149080	6.00×10^{-21}	23233662
rs12367888	Monocyte count	rs113681054	3.00×10^{-10}	32888493
rs12367888	Monocyte percentage of white cells	rs11045886	4.00×10^{-10}	32888494
rs12367888	Neutrophil percentage of white cells	rs12317268	4.00×10^{-11}	32888494
rs12367888	Plasma estrone conjugates levels in resected early stage estrogen-receptor positive breast cancer	rs4149056	4.00×10^{-11}	28429243

rs12367888	Response to statins (LDL cholesterol percent change)	rs58310495	7.00×10^{-12}	31969989
rs12367888	Reticulocyte fraction of red cells	rs4149067	5.00×10^{-10}	32888494
rs12367888	Serum uric acid levels	rs4149056	9.00×10^{-10}	34594039
rs12367888	Sex hormone-binding globulin levels	rs57743625	3.00×10^{-117}	32042192
rs12367888	Statin-induced myopathy (severe)	rs4149056	3.00×10^{-09}	31220337
rs12367888	Total bilirubin levels	rs4149081	0.01×10^{-20}	33462484
rs12367888	Total testosterone levels	rs73079476	4.00×10^{-40}	32042192
rs12367888	Triglyceride levels	rs4149081	2.00×10^{-20}	33462484
rs12367888	Urate levels	rs4149056	2.00×10^{-08}	31578528
rs12367888	Urinary metabolite levels in chronic kidney disease	rs4149056	4.00×10^{-32}	31959995
rs12367888	Urinary metabolite modules (eigenmetabolites) in chronic kidney disease	rs55695203	4.00×10^{-22}	31959995
rs12367888	Vitamin D levels	rs12317268	2.00×10^{-14}	32242144
rs174592	Age-related disease	rs174547	1.00×10^{-29}	27790247
rs174592	Alanine aminotransferase levels	rs174576	1.00×10^{-09}	33339817
rs174592	Anorexia nervosa, attention-deficit/hyperactivity disorder, autism spectrum disorder, bipolar disorder, major depression, obsessive-compulsive disorder, schizophrenia, or Tourette syndrome (pleiotropy)	rs174592	6.00×10^{-09}	31835028
rs174592	Aortic valve stenosis	rs174547	3.00×10^{-08}	32186652
rs174592	Apolipoprotein A1 levels	rs174566	9.00×10^{-65}	32203549
rs174592	Apolipoprotein B levels	rs174564	6.00×10^{-95}	32203549
rs174592	Aspartate aminotransferase platelet ratio index in high alcohol intake	rs174566	2.00×10^{-08}	32561361

rs174592	Asthma	rs174584	$2.00x10^{-20}$	31669095
rs174592	Balding type 1	rs1535	$1.00x10^{-14}$	30595370
rs174592	Bipolar disorder	rs174592	$1.00x10^{-13}$	34002096
rs174592	C-reactive protein levels or HDL-cholesterol levels (pleiotropy)	rs174546	$2.00x10^{-24}$	27286809
rs174592	C-reactive protein levels or LDL-cholesterol levels (pleiotropy)	rs174574	$8.00x10^{-10}$	27286809
rs174592	C-reactive protein levels or triglyceride levels (pleiotropy)	rs174546	$5.00x10^{-27}$	27286809
rs174592	Cholelithiasis	rs174567	$1.00x10^{-11}$	34594039
rs174592	Cholesterol, total	rs174546	$3.00x10^{-37}$	24097068
rs174592	Chronic inflammatory diseases (ankylosing spondylitis, Crohn's disease, psoriasis, primary sclerosing cholangitis, ulcerative colitis) (pleiotropy)	rs174535	$2.00x10^{-11}$	26974007
rs174592	Colorectal cancer	rs174537	$9.00x10^{-21}$	24836286
rs174592	Comprehensive strength and appendicular lean mass	rs174547	$2.00x10^{-07}$	22960237
rs174592	Crohn's disease	rs174537	$2.00x10^{-12}$	26192919
rs174592	Delta-5 desaturase activity response to n3-polyunsaturated fat supplement	rs174566	$1.00x10^{-12}$	29246731
rs174592	Delta-6 desaturase activity	rs174545	$5.00x10^{-37}$	26584805
rs174592	Electrocardiographic traits (multivariate)	rs174537	$1.00x10^{-11}$	32602732
rs174592	Eosinophil counts	rs174583	$4.00x10^{-26}$	32888493
rs174592	Fasting glucose	rs174583	$3.00x10^{-22}$	34059833
rs174592	Gallstone disease	rs174567	$2.00x10^{-12}$	30504769
rs174592	Glycemic traits (pleiotropy)	rs174535	$2.00x10^{-08}$	31021400

rs174592	Glycerophospholipid levels	rs174547	$2.00x10^{-175}$	26068415
rs174592	Gondoic acid (20:1n-9) levels	rs174528	$3.00x10^{-46}$	28298293
rs174592	HDL cholesterol levels	rs174566	$1.00x10^{-174}$	32203549
rs174592	Heel bone mineral density	rs174574	$3.00x10^{-17}$	30048462
rs174592	Height	rs174547	$6.00x10^{-18}$	25429064
rs174592	Hematocrit	rs174578	$1.00x10^{-42}$	32888493
rs174592	Hemoglobin concentration	rs174528	$3.00x10^{-61}$	32888493
rs174592	High light scatter reticulocyte count	rs102275	$3.00x10^{-11}$	32888494
rs174592	Homeostasis model assessment of beta-cell function	rs174550	$5.00x10^{-13}$	20081858
rs174592	Hypothyroidism	rs174599	$4.00x10^{-11}$	30595370
rs174592	Inflammatory bowel disease	rs1535	$3.00x10^{-09}$	26192919
rs174592	Iron status biomarkers (total iron binding capacity)	rs174546	$7.00x10^{-22}$	33536631
rs174592	Irritable mood	rs102275	$1.00x10^{-08}$	29500382
rs174592	LDL cholesterol levels	rs174564	$3.00x10^{-48}$	32203549
rs174592	Lipid metabolism phenotypes	rs174547	$8.00x10^{-262}$	22286219
rs174592	Liver enzyme levels (alkaline phosphatase)	rs174564	$3.00x10^{-130}$	33972514
rs174592	Lysophosphatidylethanolamine levels	rs174584	$3.00x10^{-15}$	31551469
rs174592	Major depressive disorder	rs102275	$3.00x10^{-17}$	34045744
rs174592	Male-pattern baldness	rs174592	$7.00x10^{-22}$	30573740
rs174592	Mean corpuscular volume	rs174564	$2.00x10^{-34}$	32888493
rs174592	Metabolic syndrome	rs1535	$3.00x10^{-31}$	31589552
rs174592	Metabolite levels	rs102275	$4.00x10^{-264}$	22916037
rs174592	Nasal polyps	rs174535	$4.00x10^{-09}$	30643255
rs174592	Oleic acid (18:1n-9) levels	rs102275	$1.00x10^{-39}$	28298293
rs174592	Osteoporosis-related phenotypes (MTAG)	rs174547	$5.00x10^{-07}$	32107650

rs174592	P wave duration	rs174577	$3.00x10^{-08}$	24850809
rs174592	Palmitoleic acid (16:1n-7) levels	rs102275	$2.00x10^{-16}$	28298293
rs174592	Phosphatidylcholine levels	rs1535	$2.00x10^{-43}$	31551469
rs174592	Plasma omega-6 polyunsaturated fatty acid levels (arachidonic acid)	rs174547	$0.00x10^{+00}$	24823311
rs174592	Platelet count	rs174546	$9.00x10^{-20}$	34594039
rs174592	Pulse pressure	rs174564	$6.00x10^{-10}$	30578418
rs174592	QRS duration	rs174577	$4.00x10^{-11}$	27659466
rs174592	Red blood cell fatty acid levels	rs174601	$3.00x10^{-305}$	25500335
rs174592	Respiratory diseases	rs174535	$3.00x10^{-13}$	30595370
rs174592	Response to statin therapy	rs1535	$7.00x10^{-06}$	20339536
rs174592	Resting heart rate	rs174536	$2.00x10^{-30}$	27798624
rs174592	Serum alkaline phosphatase levels	rs174567	$2.00x10^{-88}$	34594039
rs174592	Serum docosahexaenoic fatty acid concentration in metabolic syndrome	rs174547	$6.00x10^{-12}$	31991592
rs174592	Serum metabolite ratios in chronic kidney disease	rs102275	$2.00x10^{-82}$	29545352
rs174592	Serum omega-6 to omega-3 polyunsaturated fatty acid ratio in metabolic syndrome	rs174547	$4.00x10^{-15}$	31991592
rs174592	Serum total protein level	rs174577	$1.00x10^{-12}$	34594039
rs174592	Sex hormone-binding globulin levels adjusted for BMI	rs174533	$1.00x10^{-28}$	32042192
rs174592	Spherical equivalent	rs174535	$3.00x10^{-10}$	32352494
rs174592	Sphingolipid levels	rs174547	$5.00x10^{-12}$	26068415
rs174592	Stearic acid (18:0) levels	rs102275	$1.00x10^{-20}$	23362303
rs174592	Stem cell factor levels	rs174528	$9.00x10^{-16}$	33067605
rs174592	Total bilirubin levels	rs174574	$8.00x10^{-20}$	34594039

rs174592	Total cholesterol levels	rs174545	$5.00x10^{-81}$	34594039
rs174592	Trans fatty acid levels	rs174574	$3.00x10^{-14}$	25646338
rs174592	Triglyceride levels	rs174566	$2.00x10^{-120}$	32203549
rs174592	Urate levels	rs174594	$5.00x10^{-08}$	31578528
rs174592	Vaccenic acid (18:1n-7) levels	rs174528	$7.00x10^{-10}$	28298293
rs174592	Vitamin C levels	rs174547	$4.00x10^{-08}$	33203707
rs28468623	Glycated hemoglobin levels	rs2301889	$8.00x10^{-06}$	34059833
rs28468623	Red cell distribution width	rs2301889	$3.00x10^{-09}$	30595370
rs28468623	Metabolite levels	rs17161692	$6.00x10^{-32}$	23093944
rs28468623	Lymphocyte counts	rs73395580	$2.00x10^{-22}$	32888493
rs28468623	Neutrophil percentage of white cells	rs3779354	$2.00x10^{-15}$	32888494
rs34367065	Acenocoumarol maintenance dosage	rs12772169	$8.00x10^{-12}$	19578179
rs34367065	Warfarin maintenance dose (adjusted for clinical factors)	rs12772169	$8.00x10^{-10}$	28686080
rs34367065	Serum alkaline phosphatase levels	rs34870400	$2.00x10^{-10}$	33547301
rs34367065	Clopidogrel active metabolite levels	rs7915414	3.00x10-14	28207573
rs4663971	L1-L4 bone mineral density x serum urate levels interaction	rs4663971	$9.00x10^{-06}$	34046847
rs4663971	Bilirubin levels in tenofovir-treated HIV infection	rs10929301	$7.00x10^{-09}$	26148204
rs4663971	Total bilirubin levels in HIV-1 infection	rs3755319	$9.00x10^{-20}$	25884002
rs5751777	Alanine aminotransferase levels	rs5751775	$1.00x10^{-10}$	34594039
rs5751777	Blood protein levels	rs5760120	$8.00x10^{-26}$	30072576
rs5751777	Liver enzyme levels (alkaline phosphatase)	rs5751777	$1.00x10^{-22}$	33972514
rs5751777	Macrophage Migration Inhibitory Factor levels	rs2330634	$5.00x10^{-10}$	27989323
rs5751777	Neutrophil count	rs1985951	$1.00x10^{-10}$	32888493

rs5751777	Protein quantitative trait loci (liver)	rs4822455	$3.00x10^{-71}$	32778093
rs5751777	Serum alkaline phosphatase levels	rs5751775	$8.00x10^{-17}$	33547301
rs5751777	Triglyceride levels	rs140288	$4.00x10^{-11}$	32203549
rs58231493	Urinary metabolite levels in chronic kidney disease	rs11626972	$3.00x10^{-145}$	31959995
rs6889699	Metabolite levels	rs113590482	$5.00x10^{-23}$	33031748
rs9943251	Metabolite levels	rs6693388	$4.00x10^{-38}$	24816252
rs9943251	Acylcarnitine levels	rs10494270	$5.00x10^{-13}$	26068415
rs9943251	Metabolite concentrations in chronic kidney disease	rs6657658	$1.00x10^{-12}$	29545352

PMID: PubMed ID

Table S6. Pathway analysis for index SNPs from SNP-nexus[2]

Index SNP	Genes Involved	Description	Parent(s)	P-value
rs10006452, rs11045856, rs12367888, rs174592, rs2277119, rs34367065, rs4663971, rs4997684, rs6889699	CYP39A1, CYP2C18, CYP2C19, UGT2B7, FADS2, SLCO1B1, UGT3A1, CYP4V2, UGT1A8, UGT1A10, UGT1A9, UGT1A7, UGT1A6, UGT1A5, UGT1A4, UGT1A3, UGT1A1	Metabolism	Metabolism	<0.001
rs10006452, rs2277119, rs34367065, rs4663971, rs4997684, rs6889699	CYP39A1, CYP2C18, CYP2C19, UGT2B7, UGT3A1, CYP4V2, UGT1A8, UGT1A10, UGT1A9, UGT1A7, UGT1A6, UGT1A5, UGT1A4, UGT1A3, UGT1A1	Biological oxidations	Metabolism	<0.001
rs10006452, rs4663971, rs6889699	UGT2B7, UGT3A1, UGT1A8, UGT1A10, UGT1A9, UGT1A7, UGT1A6, UGT1A5, UGT1A4, UGT1A3, UGT1A1	Phase II - Conjugation of compounds	Metabolism	<0.001
rs10006452, rs4663971, rs6889699	UGT2B7, UGT3A1, UGT1A8, UGT1A10, UGT1A9, UGT1A7,	Glucuronidation	Metabolism	<0.001

	UGT1A6, UGT1A5, UGT1A4, UGT1A3, UGT1A1			
rs11045856, rs12367888	SLCO1B1	Defective SLCO1B1 causes hyperbilirubinemia, Rotor type (HBLRR)	Disease	0.002
rs11045856, rs12367888	SLCO1B1	Recycling of bile acids and salts	Metabolism	0.028
rs11045856, rs12367888	SLCO1B1	Transport of organic anions	Transport of small molecules	0.021
rs11045856, rs12367888, rs174592, rs2277119, rs34367065, rs4663971	CYP39A1, CYP2C19, FADS2, SLCO1B1, UGT1A9	Metabolism of lipids	Metabolism	0.008
rs11045856, rs12367888, rs2277119	CYP39A1, SLCO1B1	Bile acid and bile salt metabolism	Metabolism	0.003
rs11045856, rs12367888, rs2277119	CYP39A1, SLCO1B1	Metabolism of steroids	Metabolism	0.029
rs11045856, rs12367888, rs4663971	SLCO1B1, UGT1A4	Heme degradation	Metabolism	<0.001

rs11045856, rs12367888, rs4663971	SLCO1B1, UGT1A4	Metabolism of porphyrins	Metabolism	0.001
rs174592	FADS2	alpha-linolenic (omega3) and linoleic (omega6) acid metabolism	Metabolism	0.023
rs174592, rs34367065	CYP2C19, FADS2	Fatty acid metabolism	Metabolism	0.039
rs2035742	HHIP	GLI proteins bind promoters of Hh responsive genes to promote transcription	Signal Transduction	0.012
rs2035742	HHIP	Ligand-receptor interactions	Signal Transduction	0.014
rs2277119	CYP39A1	Synthesis of bile acids and bile salts via 24-hydroxycholesterol	Metabolism	0.025
rs2277119, rs34367065, rs4997684	CYP39A1, CYP2C18, CYP2C19, CYP4V2	Cytochrome P450 - arranged by substrate type	Metabolism	<0.001
rs2277119, rs34367065, rs4997684	CYP39A1, CYP2C18, CYP2C19, CYP4V2	Phase I - Functionalization of compounds	Metabolism	<0.001
rs2277119, rs4997684	CYP39A1, CYP4V2	Endogenous sterols	Metabolism	0.001
rs34367065	CYP2C18, CYP2C19	Xenobiotics	Metabolism	0.001
rs34367065	CYP2C19	Synthesis of epoxy (EET) and dihydroxyeicosatrienoic acids (DHET)	Metabolism	0.014
rs34367065	CYP2C19	Synthesis of (16-20)-hydroxyeicosatetraenoic acids (HETE)	Metabolism	0.016

rs34367065	CYP2C19	CYP2E1 reactions	Metabolism	0.02
rs4663971	UGT1A8	Defective UGT1A1 causes hyperbilirubinemia	Disease	0.002
rs4663971	UGT1A4	Defective UGT1A4 causes hyperbilirubinemia	Disease	0.002
rs4663971	UGT1A8, UGT1A4	Metabolic disorders of biological oxidation enzymes	Disease	0.002
rs4663971	UGT1A3	NR1H2 & NR1H3 regulate gene expression to control bile acid homeostasis	Signal Transduction	0.016
rs4997684	CYP4V2	The canonical retinoid cycle in rods (twilight vision)	Signal Transduction	0.039

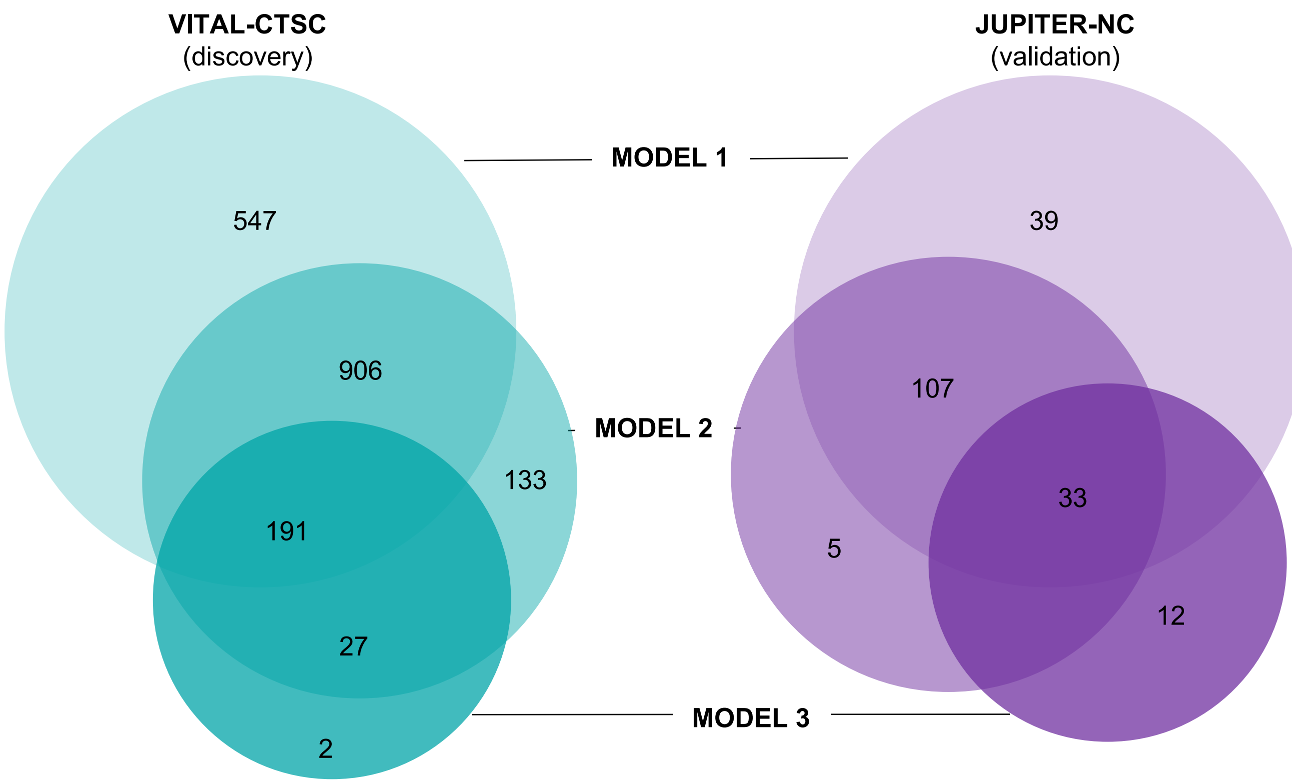
Table S7. Results from mediation analysis through BALs in the relationship between PA and CVD in VITAL-CVD sub-study with validation in JUPITER-CVD.

		VITAL-CVD						JUPITER-CVD					
BAL m/z	**rt**	**Indirect Effect**			**Direct effect**			**Indirect Effect**			**Direct effect**		
		Estimate	95% CI	P	Estimate	95% CI	P	Estimate	95% CI	P	Estimate	95% CI	P
341.2699	**3.74**	-0.048	-0.076,-0.019	0.001	-0.016	-0.13,0.098	0.786	-0.009	-0.022,0.004	0.155	-0.058	-0.135,0.018	0.135
599.3444	**1.86**	-0.031	-0.056,-0.006	0.013	-0.031	-0.144,0.083	0.595	-0.005	-0.014,0.003	0.245	-0.063	-0.139,0.013	0.103
613.3571	**1.99**	-0.027	-0.048,-0.005	0.014	-0.03	-0.143,0.084	0.61	-0.016	-0.031,-0.001	0.042	-0.057	-0.134,0.019	0.14
613.3599	**2.06**	-0.028	-0.05,-0.006	0.014	-0.029	-0.143,0.084	0.612	-0.016	-0.031,-0.001	0.042	-0.057	-0.134,0.019	0.14
611.3807	**3.34**	-0.026	-0.047,-0.004	0.019	-0.034	-0.147,0.079	0.557	-0.004	-0.012,0.004	0.311	-0.066	-0.142,0.01	0.09
611.3807	**3.46**	-0.024	-0.044,-0.003	0.021	-0.038	-0.151,0.075	0.514	-0.003	-0.011,0.004	0.367	-0.067	-0.143,0.009	0.084
625.3511	**2.40**	-0.024	-0.045,-0.004	0.022	-0.035	-0.148,0.079	0.549	-0.011	-0.024,0.002	0.111	-0.064	-0.14,0.013	0.102
479.3394	**3.91**	-0.023	-0.042,-0.003	0.022	-0.031	-0.145,0.083	0.592	-0.01	-0.022,0.002	0.111	-0.061	-0.137,0.015	0.118
257.1759	**2.85**	-0.021	-0.04,-0.002	0.031	-0.039	-0.152,0.074	0.496	-0.001	-0.005,0.003	0.643	-0.067	-0.143,0.009	0.084
543.2783	**1.18**	-0.019	-0.037,-0.001	0.036	-0.036	-0.15,0.077	0.53	-0.003	-0.01,0.004	0.346	-0.064	-0.14,0.012	0.099
307.2279	**6.27**	-0.019	-0.037,-0.001	0.036	-0.04	-0.153,0.072	0.482	0.001	-0.003,0.006	0.599	-0.065	-0.141,0.011	0.092

251.1657	**4.81**	-0.02	-0.039,-0.001	0.037	-0.037	-0.15,0.077	0.525	0.001	-0.003,0.005	0.644	-0.068	-0.144,0.008	0.077
343.2856	**4.29**	-0.027	-0.052,-0.001	0.039	-0.036	-0.15,0.078	0.537	-0.003	-0.009,0.004	0.386	-0.064	-0.14,0.012	0.098
497.3496	**2.69**	-0.022	-0.043,0	0.049	-0.033	-0.147,0.081	0.571	-0.022	-0.04,-0.004	0.016	-0.048	-0.125,0.029	0.219

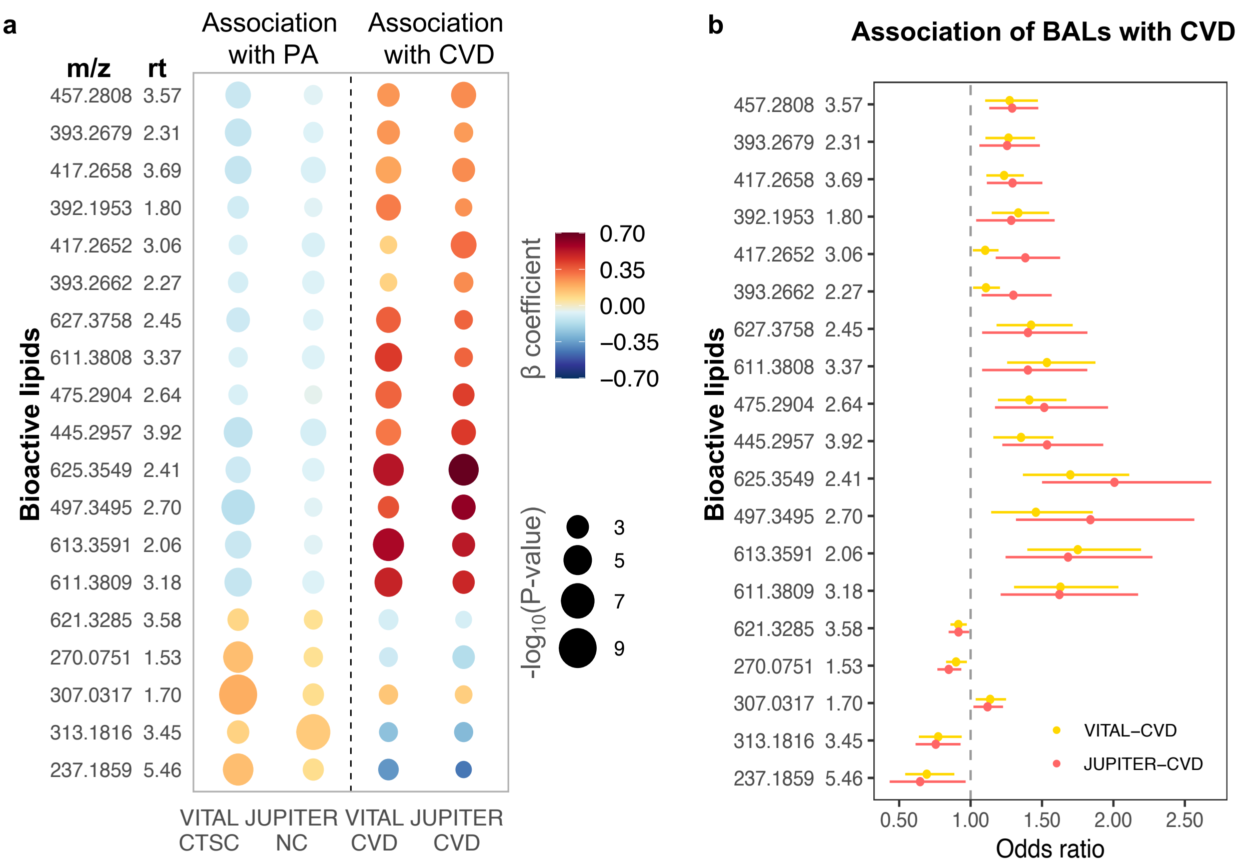
Mediation analysis adjusted for age, sex, race/ethnicity, LDL-C, total-C, and smoking; BAL – Bioactive lipid; m/z – mass to charge ratio; rt – retention time.

Fig. S1: Significant PA-BAL association counts identified by each model in both discovery and validation cohorts



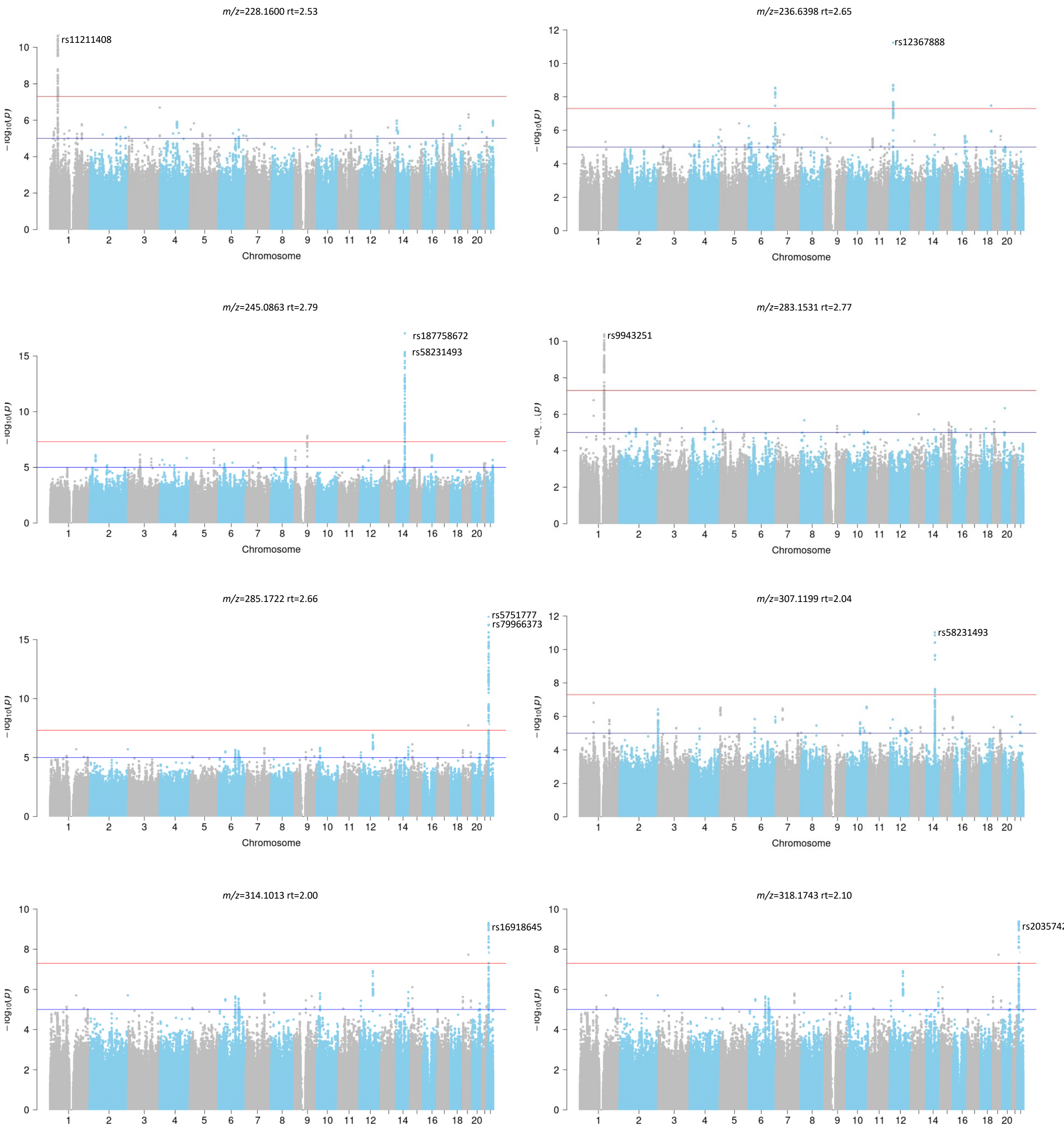
Model 1 was adjusted for age and sex.
Model 2 was adjusted for age, sex, race/ethnicity, LDL-cholesterol, total-cholesterol, and smoking.
Model 3 was adjusted for same covariates as in model 2 plus categorized BMI (< 25, 25–29.9, or ≥ 30 kg/m2) and HDL-cholesterol.

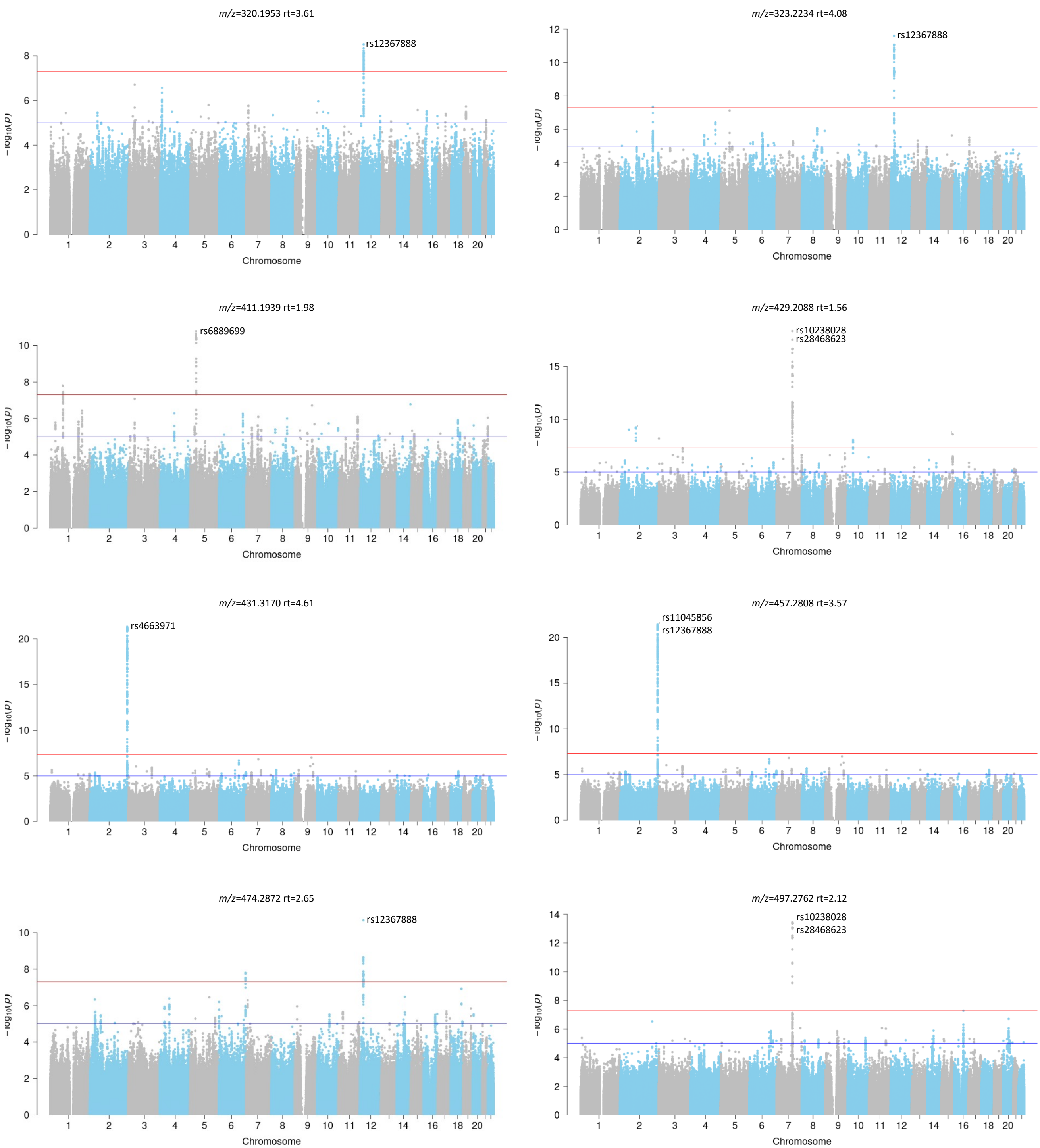
Fig. S2: Results from model 1, adjusted for age and sex



a) Rainplot representing β coefficients for the 19 BALs that associated with incident CVD (FDR < 0.1) and respective associations with PA. The dashed line separates PA-BAL (left side) from BAL-CVD (right side) associations. **b)** Odds ratio for the association of PA-significant BALs and incident CVD events. **Abbreviations**: m/z – mass to charge ratio; rt – retention time.

Fig. S3: Manhattan plots showing index SNPs within independent loci associated with PA-related BALs

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m/z=429.2088 rt=1.56
rs10238028
rs28468623
m/z=431.3170 rt=4.61
rs4663971
m/z=457.2808 rt=3.57
rs11045856
rs12367888
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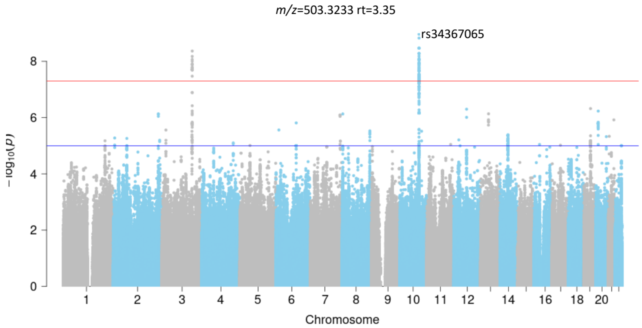
−log10(P)
Chromosome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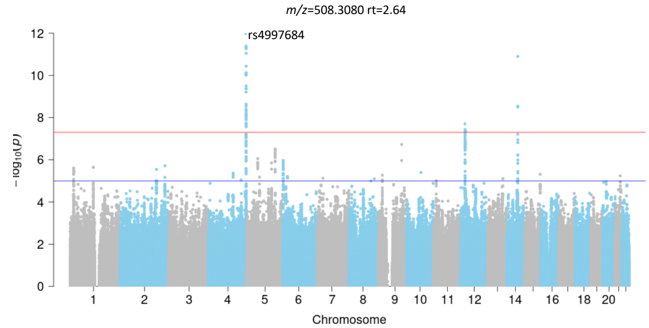
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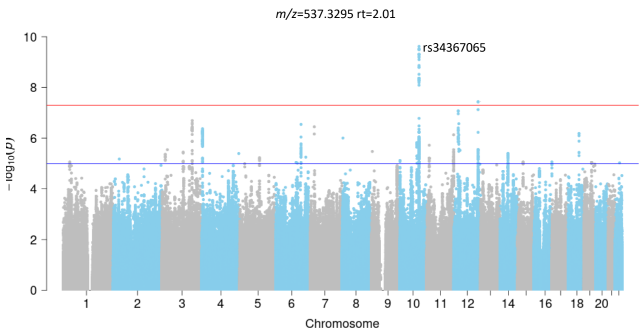
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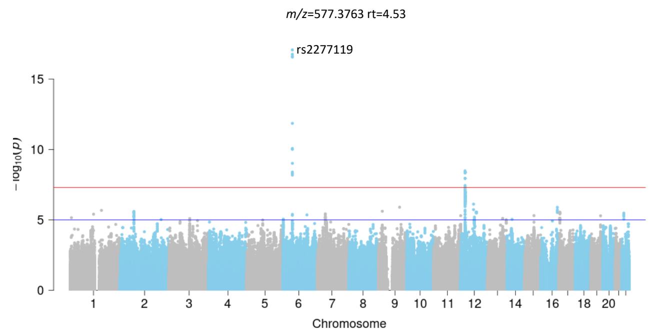
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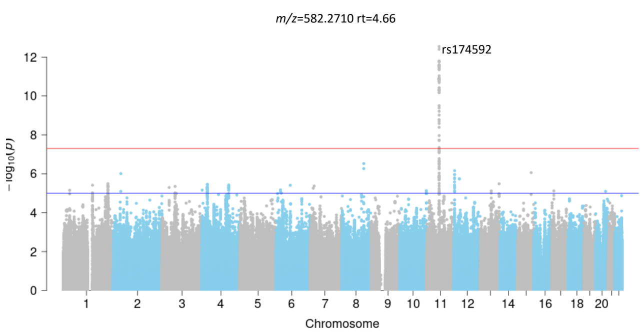
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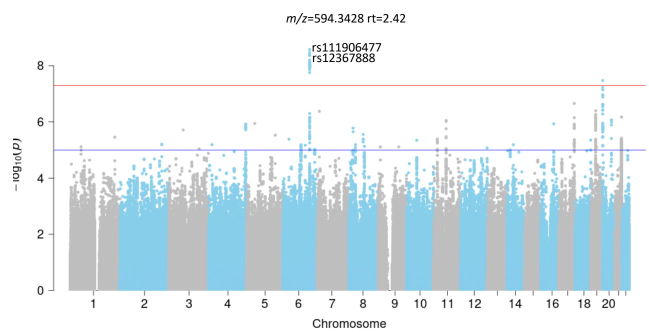
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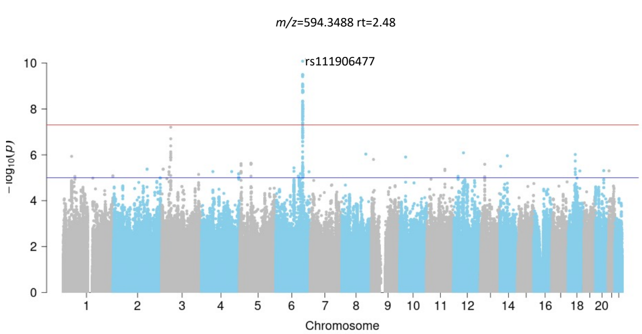
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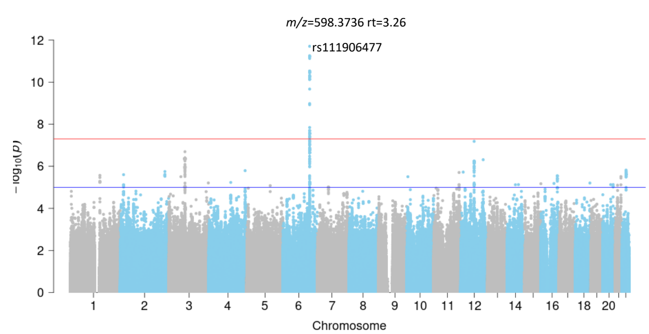
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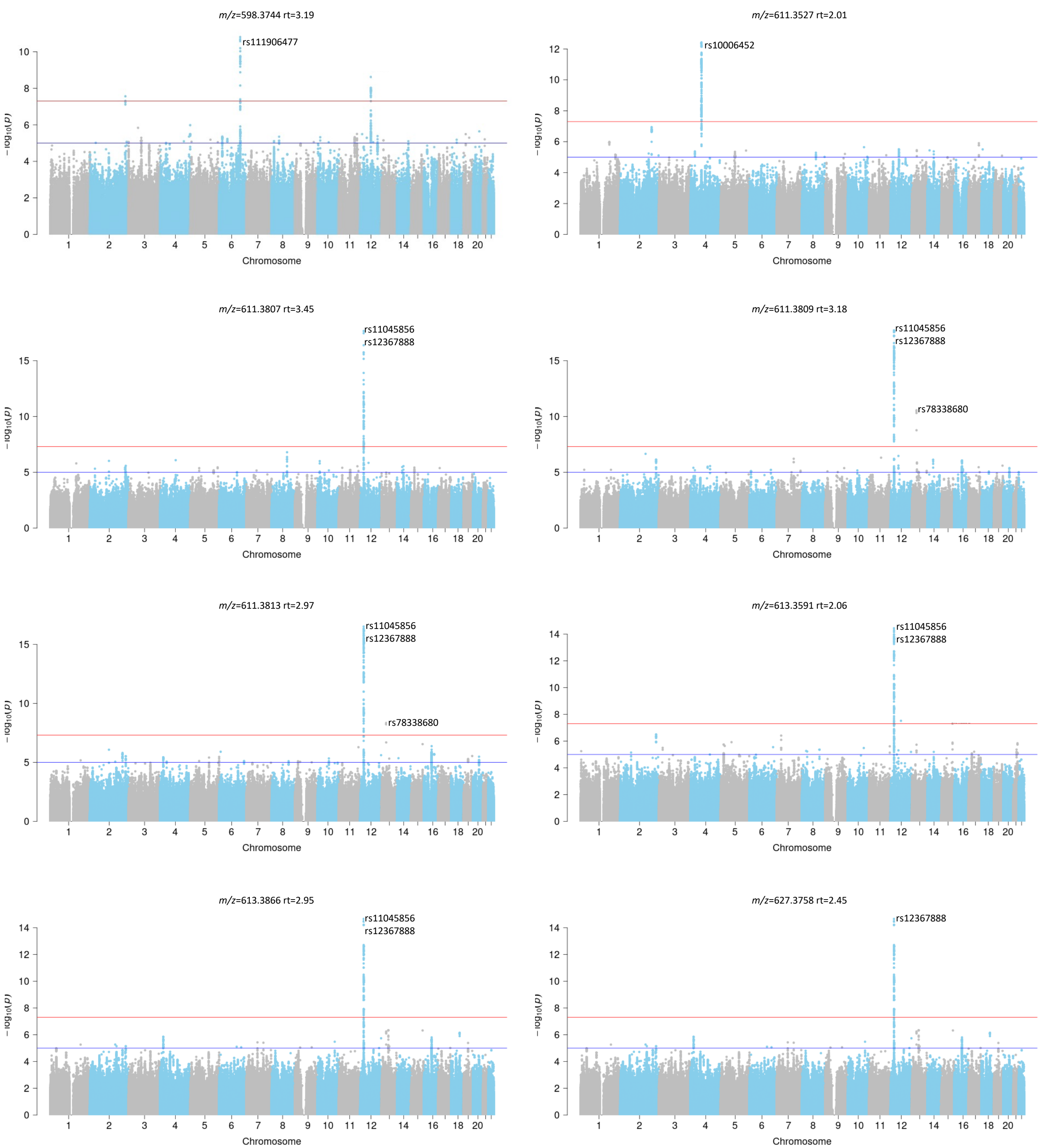
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rs12367888
m/z=611.3809 rt=3.18
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rs12367888
rs78338680
m/z=611.3813 rt=2.97
rs11045856
rs12367888
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m/z=613.3591 rt=2.06
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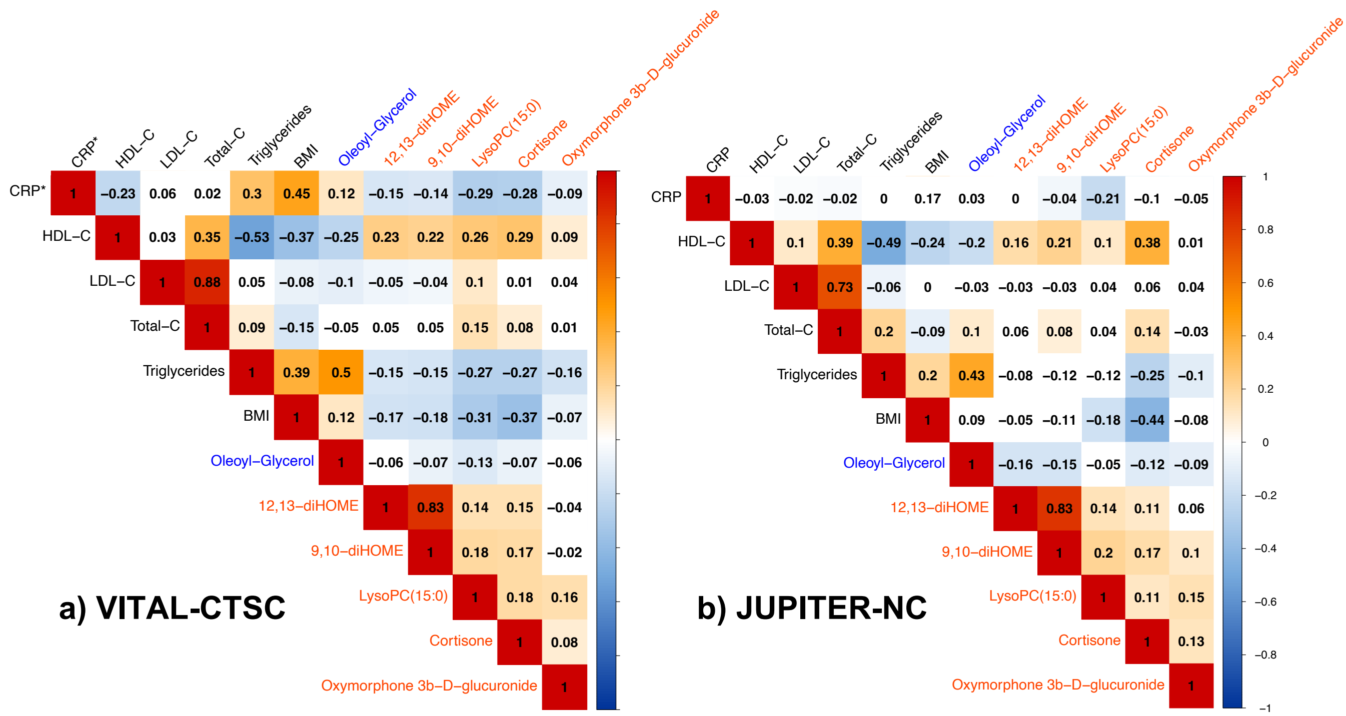
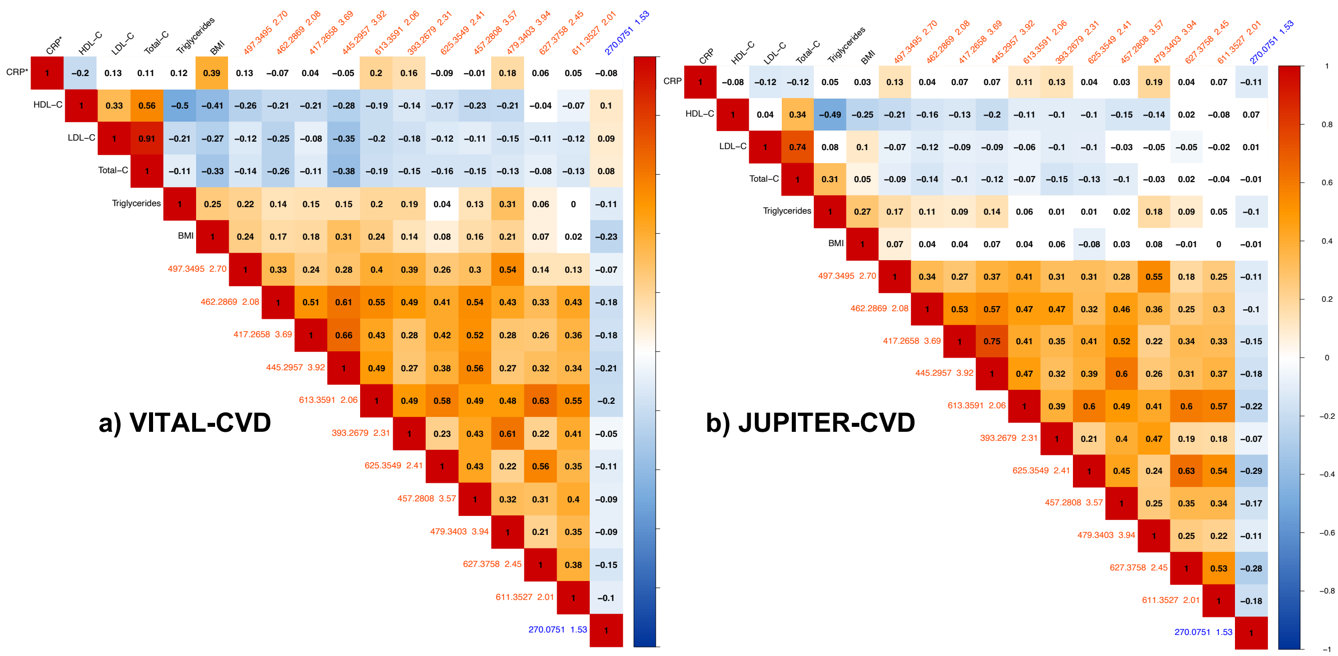
m/z=613.3866 rt=2.95
rs11045856
rs12367888
m/z=627.3758 rt=2.45
rs12367888
−log10(P)
Chromosome

Fig. S4: correlation matrices among PA significant annotated BAL, standard lipid markers, and BMI in VITAL-CTSC and JUPITER-NC substudies



a) Spearman correlation matrix in VITAL-CTSC of baseline PA-associated annotated BALs (model 2), hsCRP, clinical lipid biomarkers, and BMI. CRP* data available for 1015 participants. **b)** Spearman correlation matrix in JUPITER-NC of baseline PA-associated annotated BALs (model 2), CRP, clinical lipid biomarkers, and BMI. Black labels represent clinical measurements and biomarkers; Blue labels designate PA-negative related BAL; Orange labels designate PA-positive related BALs. The Jennrich test for the equality of two correlation matrices revealed P-value <0.001. **Abbreviations**: BMI – body mass index; LDL-C – low density lipoprotein cholesterol; HDL-C – high density lipoprotein cholesterol; hs-CRP – high-sensitivity C-reactive protein.

Fig. S5: correlation matrices among CVD significant BAL, standard lipid markers, and BMI in VITAL-CVD and JUPITER-CVD sub-studies



a) Spearman correlation matrix in VITAL-CVD of 12 CVD-associated (and validated) BALs (model 2), CRP, clinical lipid biomarkers, and BMI. CRP* data available only for 97 cases and 89 controls. **b)** Spearman correlation matrix in JUPITER-CVD of 12 CVD-associated (and validated) BALs (model 2), CRP, clinical lipid biomarkers, and BMI. Black labels represent clinical measurements and biomarkers; Orange labels designate CVD-positive related BAL. Blue label designate CVD-negative related BAL. Labels numbers are mass to charge ratio and retention time (*m/z* rt). The Jennrich test for the equality of two correlation matrices revealed P-value <0.001. **Abbreviations**: BMI – body mass index; LDL-C – low density lipoprotein cholesterol; HDL-C – high density lipoprotein cholesterol; hs-CRP – high-sensitivity C-reactive protein.

Fig. S6: Mediation analysis through BMI in the relationship between PA and BALs and between BALs and CVD

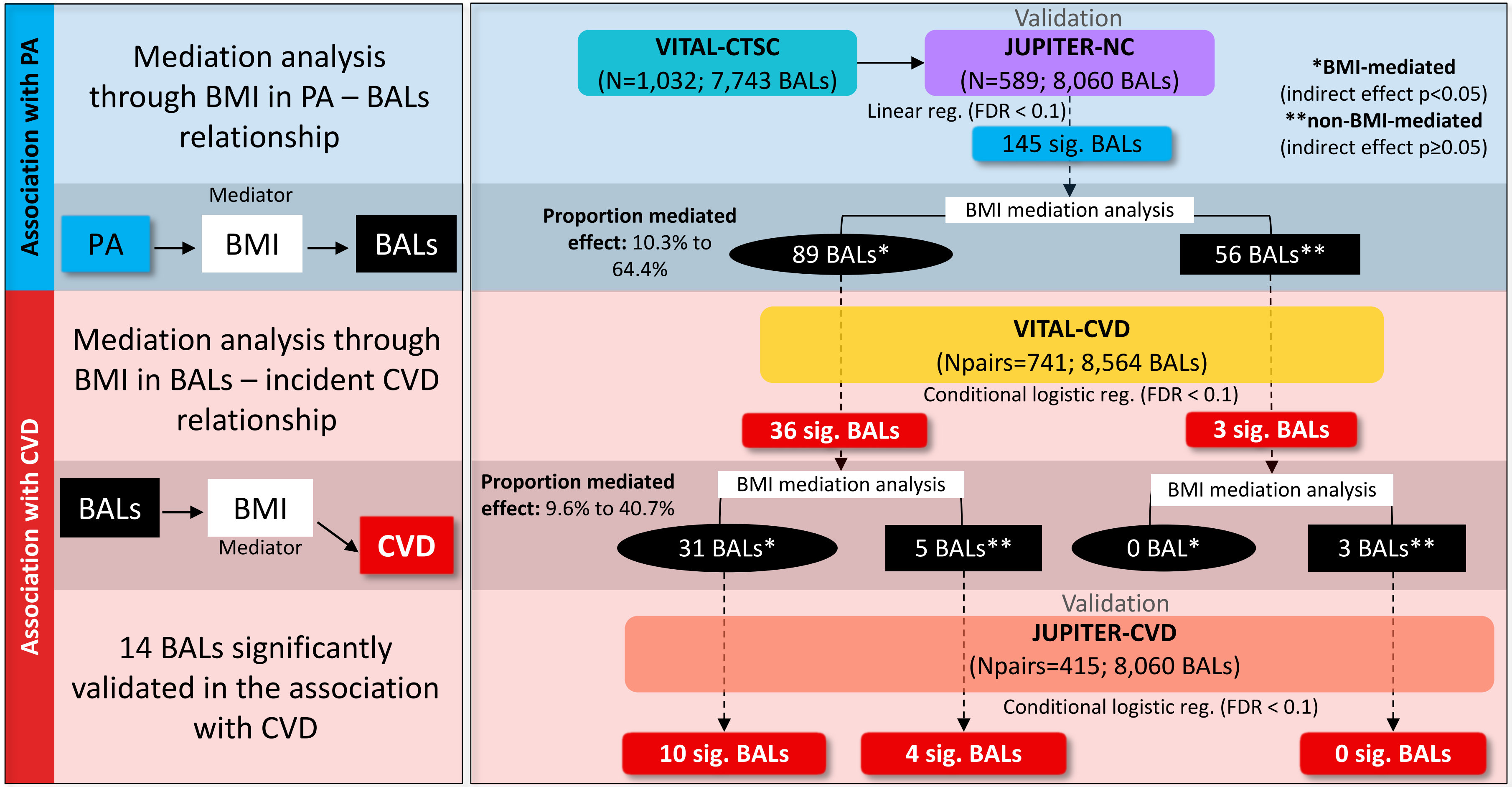


Diagram showing BAL associations mediated and non-mediated by BMI (adjusted for sex, age, race, LDL-C, total-C, and smoking). **Top blue panel**: Mediation analysis through baseline BMI performed on 145 cross-sectionally PA-related BAL revealed two sets of metabolites based on direct and indirect effects: 89 BMI-mediated and 56 non-BMI-mediated BALs. **Bottom red panel**: Each set of BALs was examined for associations with CVD and mediation through BMI. From the PA-BMI mediated set, we detected 32 BMI-mediated CVD associations in VITAL-CVD of which 11 validated in JUPITER-CVD; 4 non-mediated CVD associations in VITAL-CVD were also validated in JUPITER-CVD. From the set of non-mediated PA-BAL, no CVD association was validated in JUPITER-CVD. *indirect effect P-value < 0.05; **indirect effect P-value ≥ 0.05. Since no association between BMI and incident CVD was found in JUPITER-CVD, mediation analysis was not performed at this stage.

Fig. S7: Mediation analysis through BALs in the relationship between PA and CVD

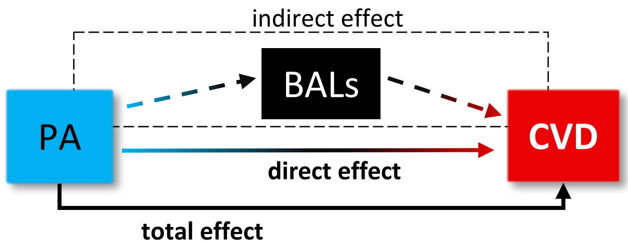


Diagram representing schematically the calculation of results presented in Table S7 from mediation analysis performed between PA and CVD through BALs. Indirect effect: the impact of PA on the CVD through BALs. Direct effect: the impact of PA on CVD in the presence of BALs as mediators. Total effect: the impact of PA on CVD without the involvement of BALs as mediators.