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Supplemental information

Cold-induced changes in plasma signaling lipids are associated with a healthier cardiometabolic profile independently of brown adipose tissue

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Changes in plasma signaling lipids after 2 h of cold exposure are associated with a healthier cardiometabolic profile independently of brown adipose tissue in young adults

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SUPPLEMENTARY MATERIAL

Table S1. STROBE Statement—checklist of items that should be included in reports of observational studies. Related to Table 1 and Figures 1-4.

	Item No.	Recommendation	Page No.
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	22-23
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	22
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	21,22
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	23
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	23-26
Bias	9	Describe any efforts to address potential sources of bias	23-26
Study size	10	Explain how the study size was arrived at	26
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	26
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	26-28
		(*b*) Describe any methods used to examine subgroups and interactions	26-28
		(*c*) Explain how missing data were addressed	26-28
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	N/A

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	26-28
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure S1
		(b) Give reasons for non-participation at each stage	Figure S1
		(c) Consider use of a flow diagram	Figure S1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	6
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6-9
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	10-15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Table S2. 2017 CONSORT checklist of information to include when reporting a randomized trial assessing nonpharmacologic treatments (NPTs). Related to Figure 5.

Section/Topic Item	Checklist item no.	CONSORT item	Extension for NPT trials	Reported on page nº
Title and abstract				
	1a	Identification as a randomized trial in the title		*N/A*
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	*Refer to CONSORT extension for abstracts for NPT trials*	*N/A*
Introduction				
Background and objectives	2a	Scientific background and explanation of rationale		3-4
	2b	Specific objectives or hypotheses		5
Methods				
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	When applicable, how care providers were allocated to each trial group	22
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons		22
Participants	4a	Eligibility criteria for participants	When applicable, eligibility criteria for centers and for *care providers*	21
	4b	Settings and locations where the data were collected		22
Interventions†	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	Precise details of both the experimental treatment and comparator	22-23
	5a		Description of the different components of the interventions and, when applicable, description of the procedure for tailoring the interventions to individual participants.	22-23
	5b		Details *of whether and* how the interventions were standardized.	22-23
	5c.		Details *of whether and* how adherence of care providers to the protocol was assessed or enhanced	22-23
	5d		*Details of whether and how adherence of participants to interventions was assessed or enhanced*	22-23
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed		23-26
	6b	Any changes to trial outcomes after the trial commenced, with reasons		23
Sample size	7a	How sample size was determined	When applicable, details of whether and how the clustering by care providers or centers was addressed	26

Section/Topic Item	Checklist item no.	CONSORT item	Extension for NPT trials	Reported on page n°
	7b	When applicable, explanation of any interim analyses and stopping guidelines		N/A
Randomization:				
- Sequence generation	8a	Method used to generate the random allocation sequence		22-23
	8b	Type of randomization; details of any restriction (such as blocking and block size)		22-23
- Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned		22-23
- Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions		22-23
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	~~Whether or not those administering co-interventions were blinded to group assignment~~ If done, who was blinded after assignment to interventions (e.g., participants, care providers, *those administering co-interventions*, those assessing outcomes) and how	22-23
	11b	If relevant, description of the similarity of interventions	~~If blinded, method of blinding and description of the similarity of interventions~~	Not relevant
	11c		*If blinding was not possible, description of any attempts to limit bias*	22-23
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	When applicable, details of whether and how the clustering by care providers or centers was addressed	26-28
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses		26-28
Results				
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome	The number of care providers or centers performing the intervention in each group and the number of patients treated by each care provider or in each center	Figure S1
	13b	For each group, losses and exclusions after randomization, together with reasons		Figure S1
	13c		*For each group, the delay between randomization and the initiation of the intervention*	*N/A*
	new		Details of the experimental treatment and comparator as they were implemented	*N/A*
Recruitment	14a	Dates defining the periods of recruitment and follow-up		

Section/Topic Item	Checklist item no.	CONSORT item	Extension for NPT trials	Reported on page nº
	14b	Why the trial ended or was stopped		
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	When applicable, a description of care providers (case volume, qualification, expertise, etc.) and centers (volume) in each group.	Table 1 and S5
Numbers analyzed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups		Figure S1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)		6-9
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended		Not binary outcomes
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory		6-9
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)		None
Discussion				
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	In addition, take into account the choice of the comparator, lack of or partial blinding, and unequal expertise of care providers or centers in each group	15
Generalizability	21	Generalizability (external validity, applicability) of the trial findings	Generalizability (external validity) of the trial findings according to the intervention, comparators, patients, and care providers and centers involved in the trial	10-15
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence		10-15
Other information				
Registration	23	Registration number and name of trial registry		21
Protocol	24	Where the full trial protocol can be accessed, if available		Reference 53
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders		16

Table S4. List of internal standards used in the LC-MS/MS method, related to STAR Methods.

Abbreviation	Name (International Union of Pure and Applied Chemistry, IUPAC)
Arachidonic aacid-d_8 C20:4-w6-d_8	5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid-d8
Docosahexaenoic acid-d_5 (C22:6-w3-d_5)	4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*-docosahexaenoic acid-d5
Linoleic acid-d_4 (C18:2-w6-d_4)	9Z,12Z-octadecadienoic acid-d4
d_{11}-5-iPF_{2a}-VI	(8β)-5,9α,11α-trihydroxy-prosta-6*E*,14*Z*-dien-1-oic acid-d11
d_4-8-iso-PGE_2	9-oxo-11α,15*S*-dihydroxy-(8β)-prosta-5*Z*,13*E*-dien-1-oic acid-d4
d_4-8-iso-$PGF_{2\alpha}$	9α,11α,15*S*-trihydroxy-(8β)-prosta-5*Z*,13*E*-dien-1-oic acid-d4
d_4-PGD_2	9α,15*S*-dihydroxy-11-oxo-prosta-5*Z*,13*E*-dien-1-oic acid-d4
d_4-PGF2α	9*S*,11*R*,15*S*-trihydroxy-5*Z*,13*E*-prostadienoic acid-d4
d_9-PGE_2	9-oxo-11*R*,15*S*-dihydroxy-5*Z*,13*E*-prostadienoic acid-d9
d_4-iP$F_{2\alpha}$-IV	(8*S*)-10-[(1*R*,2*S*,3*S*,5*R*)-3,5-Dihydroxy-2-pentylcyclopentyl]-8-hydroxydeca-5,9-dienoic acid-d4
d_{11}-8,12-iso-iP$F_{2\alpha}$-VI	(12α)-5,9α,11α-trihydroxy-prosta-6*E*,14*Z*-dien-1-oic acid-d11
d_{17}-10-Nitrooleate	10-nitro,9Z,12*Z*-octadecadienoic acid-d17
d_{11}-14,15-DiHETrE	14,15-dihydroxy-5*Z*,8*Z*,11*Z*-eicosatrienoic acid-d11
d_4-9(S)-HODE	9*S*-hydroxy-10*E*,12*Z*-octadecadienoic acid-d4
d_4-LTB_4	5*S*,12*R*-dihydroxy-6*Z*,8*E*,10*E*,14*Z*-eicosatetraene-1,20-dioic acid-d4
d_4-TXB_2	9*S*,11,15*S*-trihydroxy-thromboxa-5*Z*,13*E*-dien-1-oic acid-d4
d_6-20-HETE	20-hydroxy-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid-d6
d_8-12(S)-HETE	12*S*-hydroxy-5*Z*,8*Z*,10*E*,14*Z*-eicosatetraenoic acid-d8
d_8-5(S)-HETE	5*S*-hydroxy-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid-d8
d_4-(+/-)12,13-DiHOME	12,13-dihydroxy-9*Z*-octadecenoic acid -d4
d_8-2-AG	2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycerol-d8
d_8-AEA	N-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-ethanolamine-d8
d_4-COR	11β,17,21-trihydroxypregn-4-ene-3,20-dione-d4
d_4-DHEA	N-(4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoyl)-ethanolamine-d4
d_4-LEA	N-(9Z,12Z-octadecadienoyl)-ethanolamine-d4
d_4-OEA	N-(9Z-octadecenoyl)-ethanolamine-d4
d_4-PEA	N-hexadecanoyl-ethanolamine-d4
d_3-SEA	N-(Octadecanoyl)-ethanolamine-d3
LPA C17:0	1-heptadecanoyl-2-hydroxy-sn-glycero-3-phosphate
S-1-P C17:1	2*S*-amino-4*E*-heptadecene-1,3*R*-diol, 1- (dihydrogen phosphate)
cLPA C17:0	1-heptadecanoyl-glycero-2,3-cyclic-phosphate
Spha-1-P C17:0	*D*-erythro-sphinganine-1-phosphate
Spha C17:0	2*S*-amino-1,3*R*-heptadecanediol
Spha C17:1	2*S*-amino-4*E*-heptadecene-1,3*R*-diol
PAF C16:0-d_4	1-*O*-hexadecyl-(7,7,8,8-d4)-2-*O*-acetyl-sn-glyceryl-3-phosphorylcholine
LPI C17:1	1-(10*Z*-heptadecenoyl)-2-hydroxy-sn-glycero-3-phospho-(1'-myo-inositol)
LPS C17:1	1-(10*Z*-heptadecenoyl)-2-hydroxy-sn-glycero-3-[phospho-L-serine]
LPG C17:1	1-(10*Z*-heptadecenoyl)-sn-glycero-3-phospho-(1'-rac-glycerol)
LPE C17:1	1-(10*Z*-heptadecenoyl)-sn-glycero-3-phosphoethanolamine

Table S5. Baseline characteristics of the study participants completing the exercise intervention (N=30). Related to Table 1 and Figure 5.

	Control (n=8)		**MOD-EX (n=11)**		**VIG-EX (n=11)**	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	21.6	2.5	21.5	2.2	21.6	2.5
Body composition						
BMI (kg/m^2)	21.9	2.0	24.9	4.4	25.3	4.9
Waist circumference (cm)	74.3	9.5	81.1	12.2	82.9	14.3
Lean mass (kg)	40.8	8.0	41.5	9.4	40.6	9.5
Fat mass (kg)	18.7	5.2	24.7	8.7	26.7	7.2
Fat mass (%)	30.4	7.2	35.7	8.3	38.3	4.0
VAT mass (g)	242.3	107.6	332.5	170.3	352.2	191.4
Cardiometabolic risk factors						
Glucose (mg/dL)	87.3	6.1	86.6	7.7	86.9	6.5
Insulin (µIU/mL)	6.0	3.2	8.2	2.6	7.7	4.5
HOMA-IR	1.3	0.8	1.8	0.7	1.7	1.2
Insulin glucose ratio	10.2	4.8	14.4	3.9	13.3	6.3
Total cholesterol (mg/dL)	149.6	12.1	161.5	28.0	182.8	36.7
HDL-C (mg/dL)	55.0	7.7	56.9	15.5	56.6	15.9
LDL-C (mg/dL)	82.9	13.8	89.5	19.2	102.7	24.2
Tryglicerides (mg/dL)	58.5	26.1	75.3	31.5	117.7	58.9
APOA1 (mg/dL)	149.3	13.8	168.4	57.3	164.5	37.4
APOB (mg/dL)	56.7	8.6	63.9	16.7	77.6	22.5
Leptin (µg/L)	2.9	1.2	6.2	5.2	7.3	3.7
Adiponectin (mg/L)	10.8	5.3	10.0	4.3	10.3	6.7
GTP (IU/L)	15.5	6.3	14.1	4.3	21.9	12.3
GGT (IU/L)	16.4	9.2	14.1	4.4	19.36	12.3
ALP (IU/L)	73.4	32.3	80.8	23.8	79.5	20.2
C-reactive protein (mg/L)	1.1	0.9	3.2	3.8	1.6	1.1
Brown adipose tissue						
BAT volume (mL)	60.8	58.4	83.4	70.0	62.8	62.1
BAT SUVmean	3.6	1.1	4.1	1.9	3.8	1.7
BAT SUVpeak	10.8	6.7	11.6	9.0	11.3	8.2
BAT radiodensity (HU)	-59.5	6.4	-58.8	7.4	-59.3	9.0

Data presented as mean and standard deviation (SD). *Abbreviations*: ALP, alkaline phosphatase; APOA1, apolipoprotein A1; APOB, apolipoprotein B; BAT, brown adipose tissue; BMI, body mass index; CON, control group; GGT, gamma-glutamyl transferase; GTP, glutamic pyruvic transaminase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance index; HU, Hounsfield units; LDL-C, low-density lipoprotein cholesterol; MOD-EX, moderate-intensity exercise group; SUV, standardized uptake value; VAT, visceral adipose tissue; VIG-EX, vigorous-intensity exercise group.

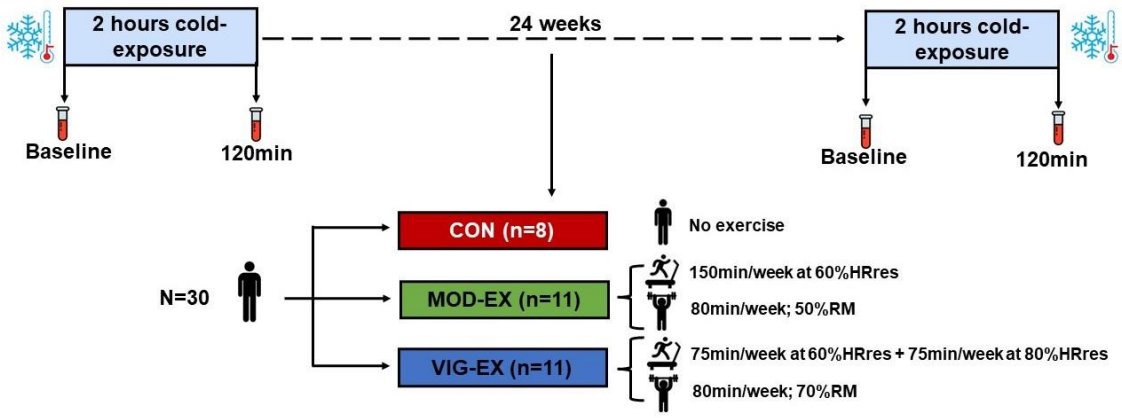

Figure S1. Design of the study investigating the influence of a 24-week supervised concurrent exercise intervention on plasma lipidome response to cold exposure in young adults. Related to Figure 1 and 5.

Abbreviations: CON, control group; HRes, heart rate reserve; min: minutes: MOD-EX, moderate-intensity exercise group; RM, repetition maximum; VIG-EX, vigorous-intensity exercise group.

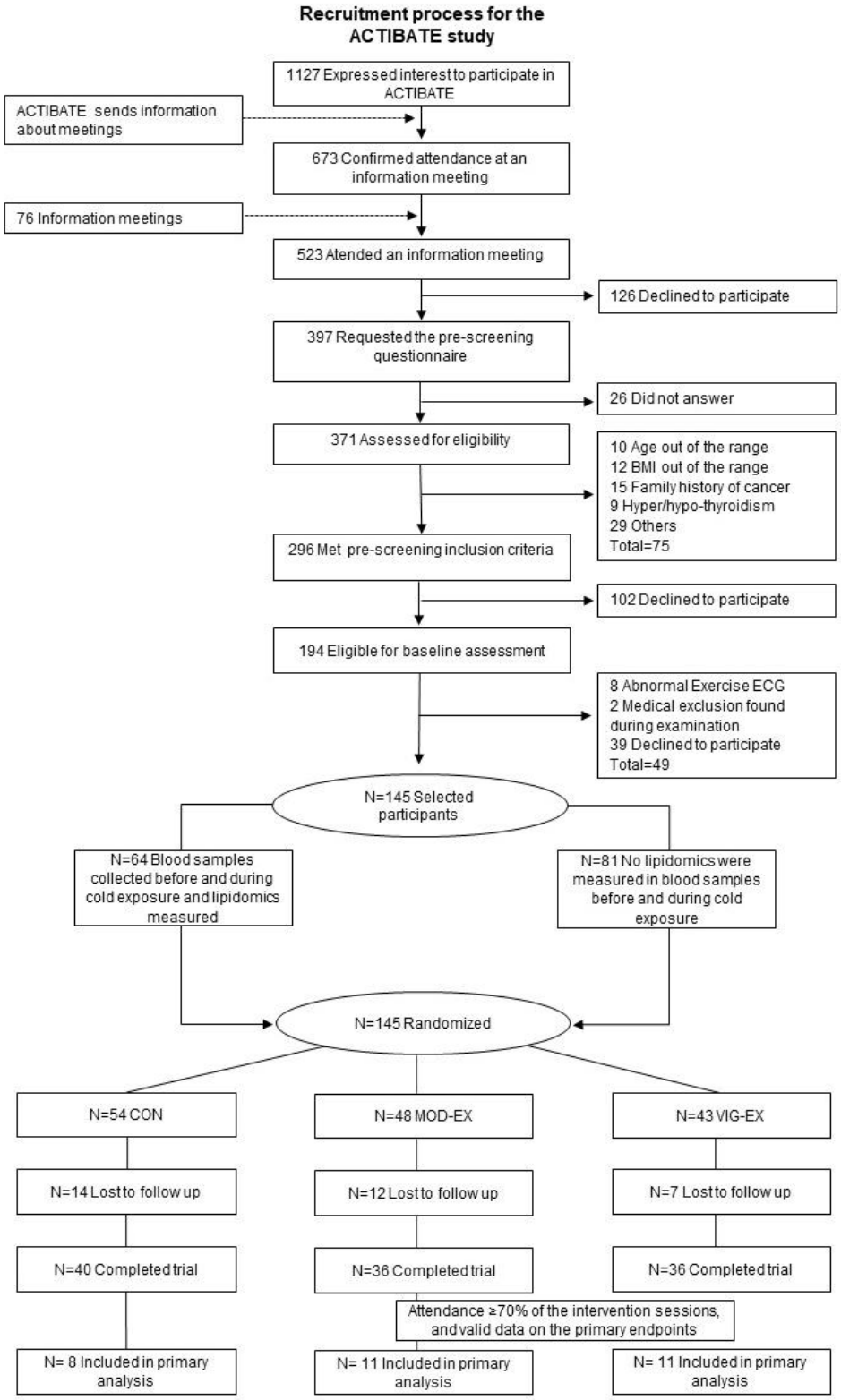

Figure S2. Study participants enrolment from the ACTIBATE study. Related to Figure 1.

Abbreviations: BMI, body mass index; CON, control group; MOD-EX, moderate-intensity exercise group; VIG-EX: vigorous-intensity exercise group; ECG, electrocardiogram.

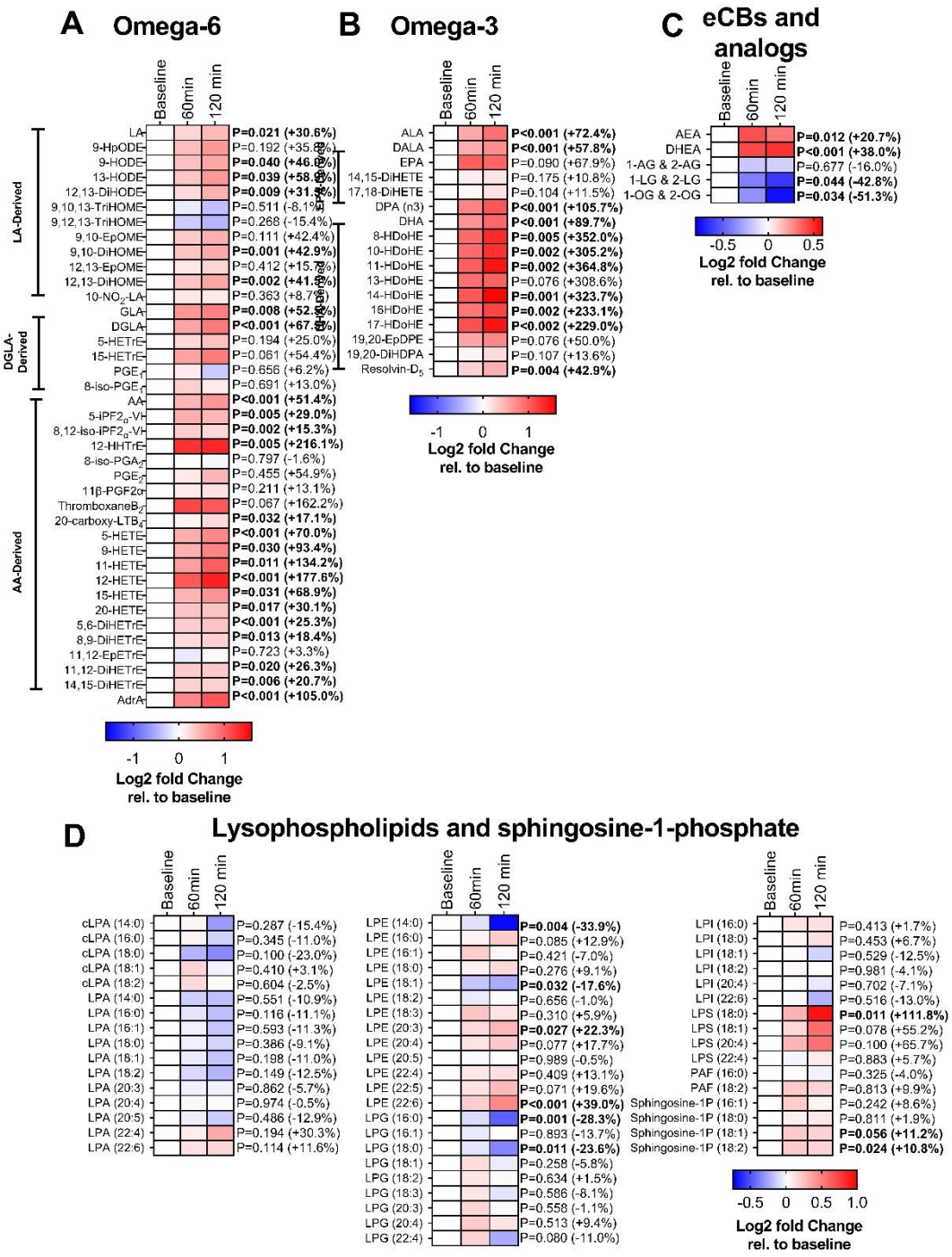

Figure S3. Effects of 2 h of cold exposure on the plasma levels of omega-6 oxylipins (A), omega-3 oxylipins (B), endocannabinoids and analogs (eCBs, C), and lysophospholipids and sphingosine-1-phosphate (D) in men (n=17). Related to Figure 2.

The color of the squares represents the mean log2 fold change of the area peak ratio of that timepoint relative to baseline. Red color represents an increase, whereas blue represents a decrease. P-values obtained from repeated measures analyses of variance (ANOVA). Values in brackets represent the mean percentage of change at 120 min relative to the baseline of each lipid. The name and abbreviations of signaling lipids are detailed in Table S3.

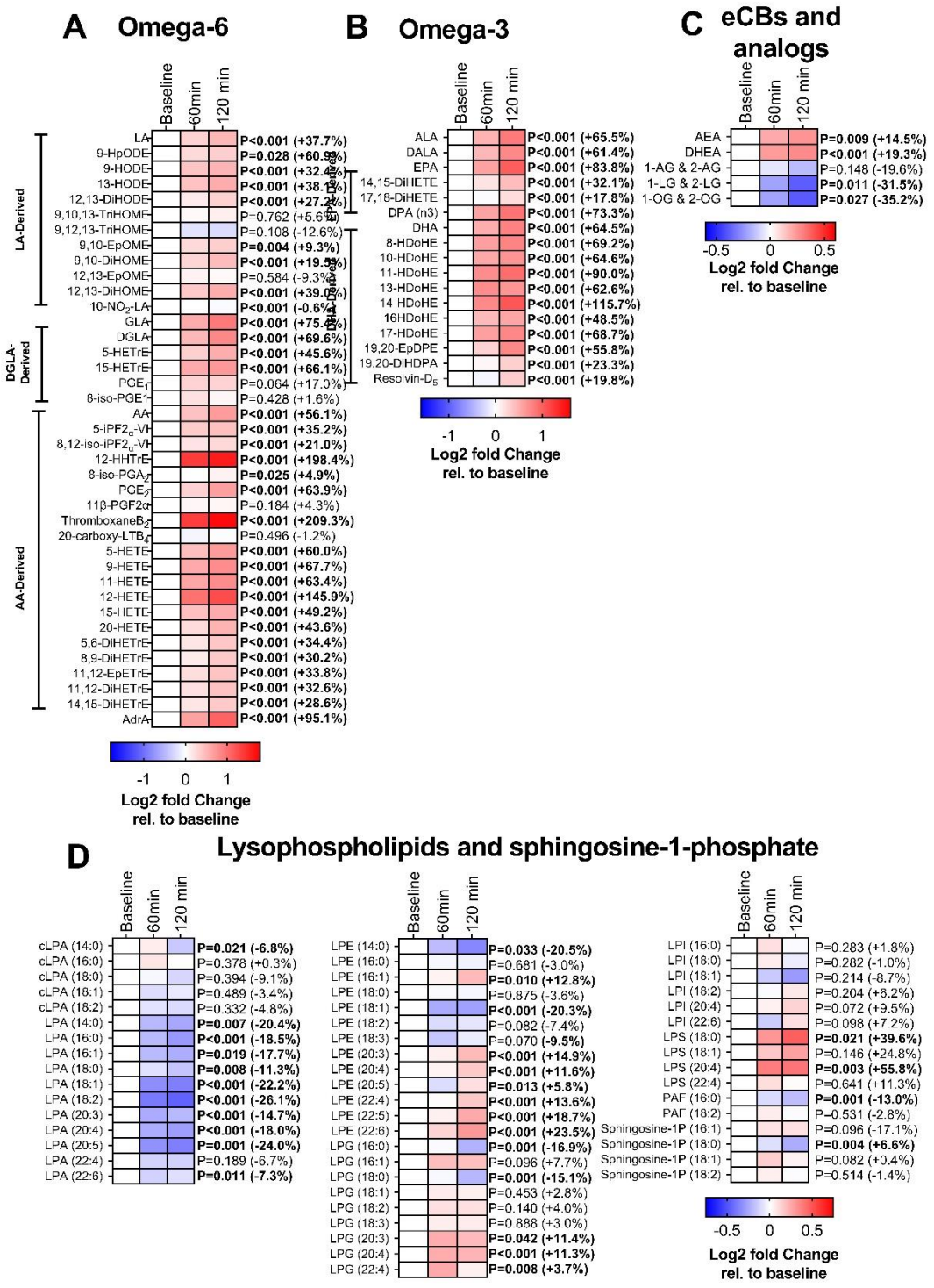

Figure S4. Effects of 2 h of cold exposure on the plasma levels of omega-6 oxylipins (A), omega-3 oxylipins (B), endocannabinoids and analogs (eCBs, C), and lysophospholipids and sphingosine-1-phosphate (D) in women (n=47). Related to Figure 2.

The color of the squares represents the mean log2 fold change of the area peak ratio of that timepoint relative to baseline. Red color represents an increase, whereas blue represents a decrease. P-values obtained from repeated measures analyses of variance (ANOVA). Values in brackets represent the mean percentage of change at 120 min relative to the baseline of each lipid. The name and abbreviations of signaling lipids are detailed in Table S3.

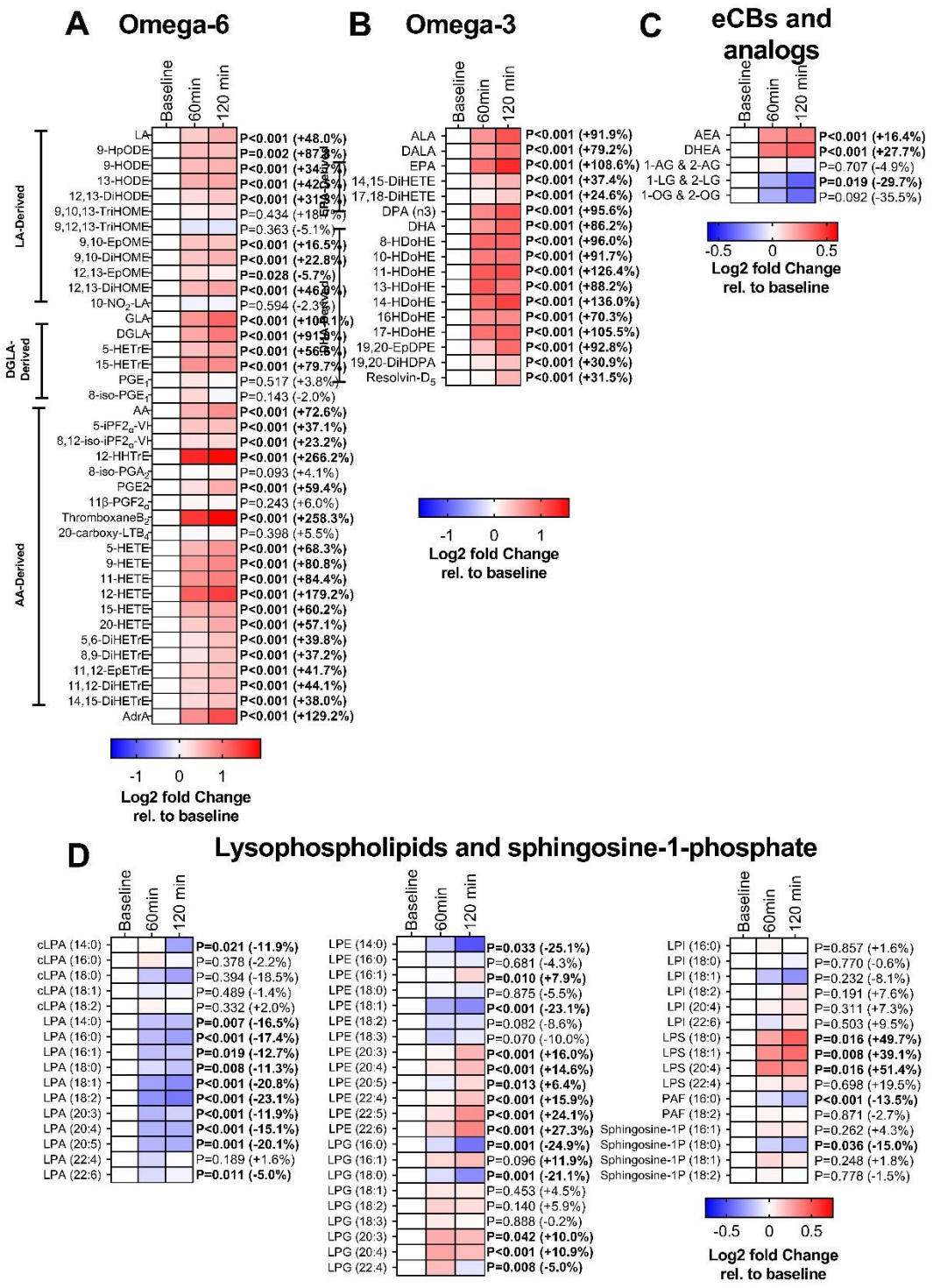

Figure S5. Effects of 2 h of cold exposure on the plasma levels of omega-6 oxylipins (A), omega-3 oxylipins (B), endocannabinoids and analogs (eCBs, C), and lysophospholipids and sphingosine-1-phosphate (D) in participants with normal weight (n=43). Related to Figure 2.

The color of the squares represents the mean log2 fold change of the area peak ratio of that timepoint relative to baseline. Red color represents an increase, whereas blue represents a decrease. P-values obtained from repeated measures analyses of variance (ANOVA). Values in brackets represent the mean percentage of change at 120 min relative to the baseline of each lipid. The name and abbreviations of signaling lipids are detailed in Table S3.

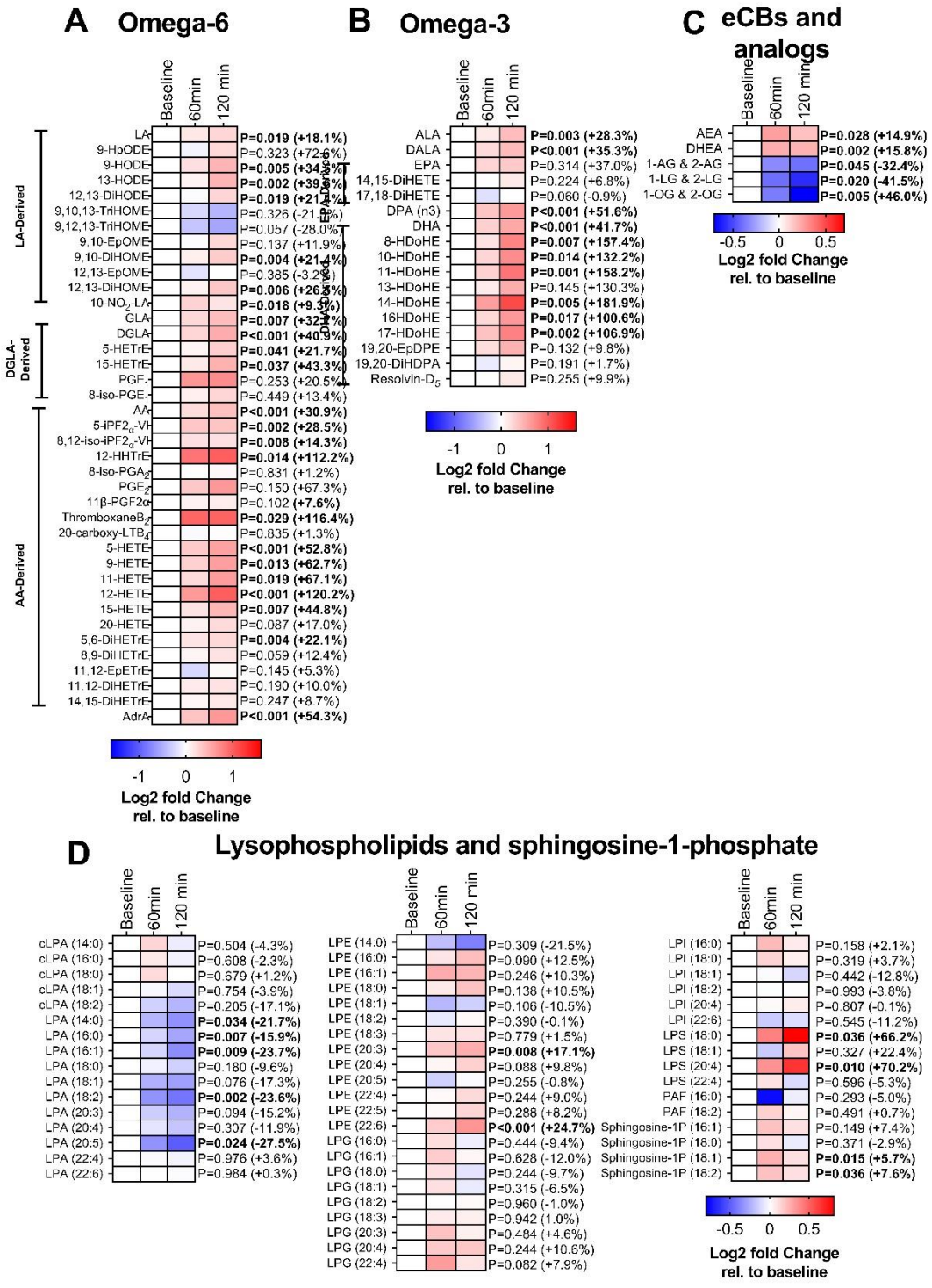

Figure S6. Effects of 2 h of cold exposure on the plasma levels of omega-6 oxylipins (A), omega-3 oxylipins (B), endocannabinoids and analogs (eCBs, C), and lysophospholipids and sphingosine-1-phosphate (D) in participants with overweight or obesity (n=21). Related to Figure 2.

The color of the squares represents the mean log2 fold change of the area peak ratio of that timepoint relative to baseline. Red color represents an increase, whereas blue represents a decrease. P-values obtained from repeated measures analyses of variance (ANOVA). Values in brackets represent the mean percentage of change at 120 min relative to the baseline of each lipid. The name and abbreviations of signaling lipids are detailed in Table S3.

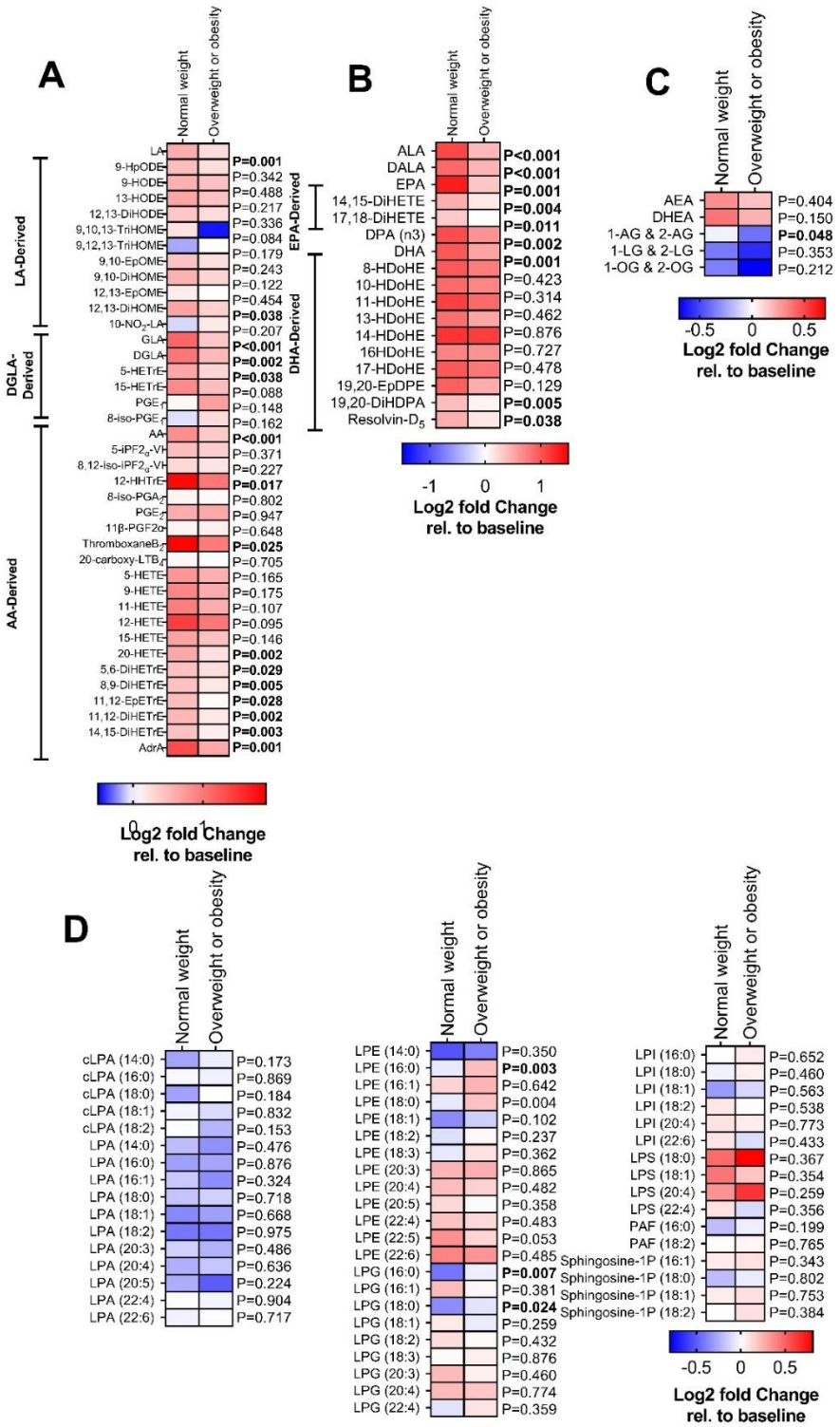

Figure S7. Differences in cold-induced changes in the plasma levels of omega-6 oxylipins (A), omega-3 oxylipins (B), endocannabinoids and analogs (eCBs, C), and lysophospholipids and sphingosine-1-phosphate sphingolipids (D) between participants with normal weight and participants with overweight or obesity. Related to Figure 2.

The color of the squares represents the mean log2 fold change of the area peak ratio of the 120 min relative to baseline. Red color represents an increase, whereas blue represents a decrease. P-values obtained from independent samples t-test.

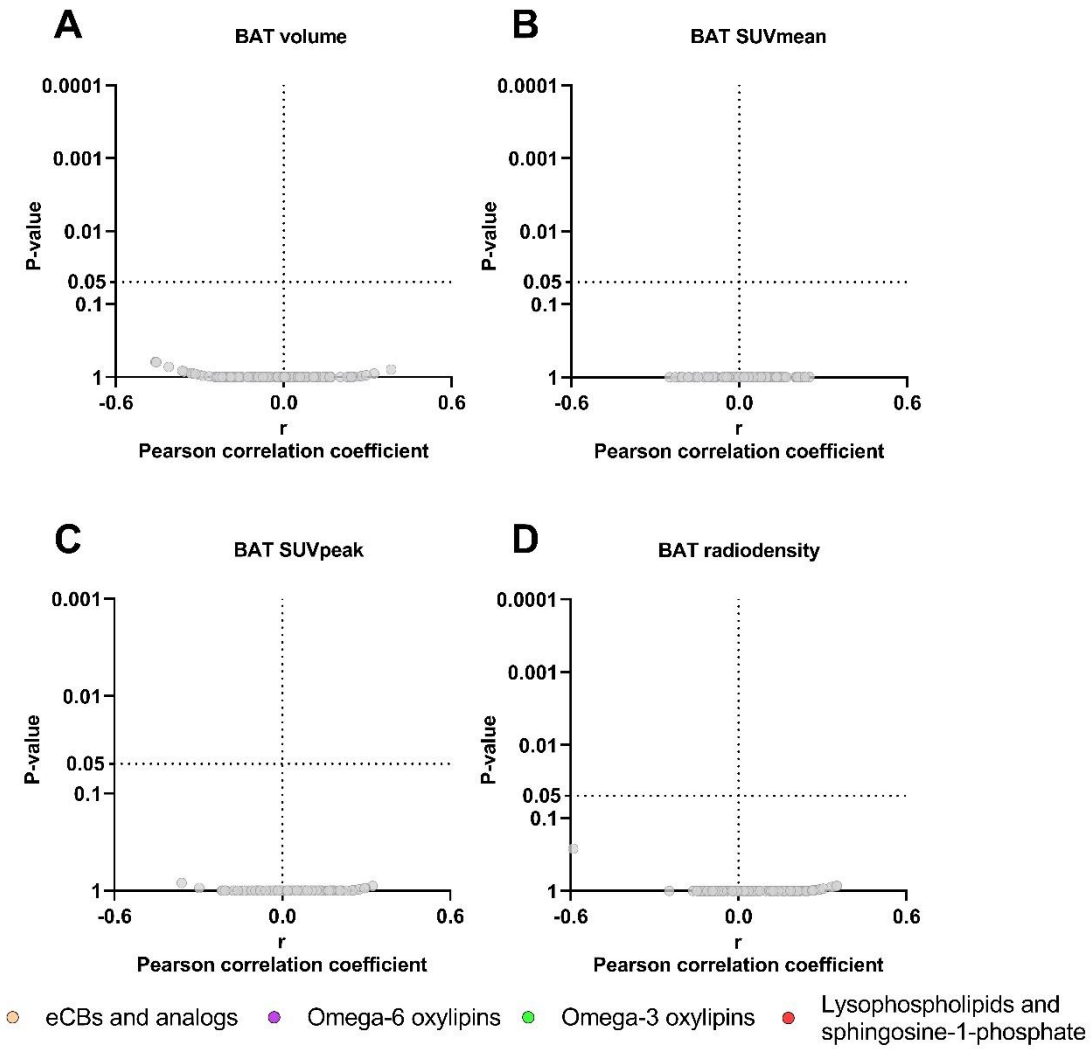

Figure S8. Association between cold-induced changes in the plasma levels of signaling lipids and brown adipose tissue-related outcomes in men (n=17). Related to Figure 3.

Volcano plots showing partial correlation analyses between the 120 min fold-change relative to baseline and BAT volume (A), BAT SUVmean (B), BAT SUVpeak (C), and BAT radiodensity (D, n=13). Partial correlation analyses were adjusted for the natural calendar day when the baseline ^{18}F-FDG-PET/CT scan was performed. The X-axis represents Pearson partial correlation coefficients, whereas the Y-axis represents the FDR-adjusted P-values of the correlations. Grey dots represent non-significant correlations, whereas colored dots represent statistically significant correlations (P-value<0.05 after FDR correction). *Abbreviations*: BAT, brown adipose tissue; eCBs, endocannabinoids; SUV, standardized uptake value.

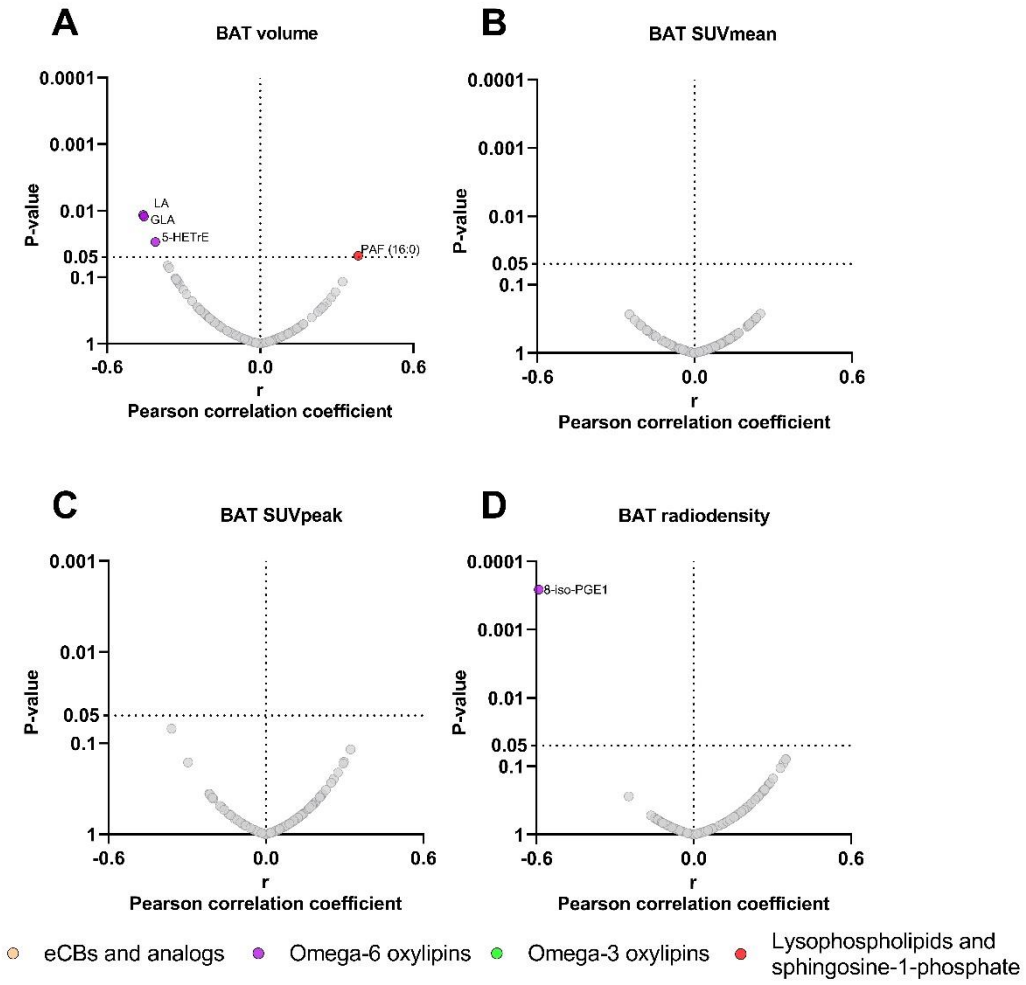

Figure S9. Association between cold-induced changes in the plasma levels of signaling lipids and brown adipose tissue-related outcomes in women (n=47). Related to Figure 3.

Volcano plots showing partial correlation analyses between the 120 min fold-change relative to baseline and BAT volume (A), BAT SUVmean (B), BAT SUVpeak (C), and BAT radiodensity (D, n=34). Partial correlation analyses were adjusted for the natural calendar day when the baseline ^{18}F-FDG-PET/CT scan was performed. The X-axis represents Pearson partial correlation coefficients, whereas the Y-axis represents the FDR-adjusted P-values of the correlations. Grey dots represent non-significant correlations, whereas colored dots represent statistically significant correlations (P-value<0.05 after FDR correction). *Abbreviations*: BAT, brown adipose tissue; eCBs, endocannabinoids; SUV, standardized uptake value.

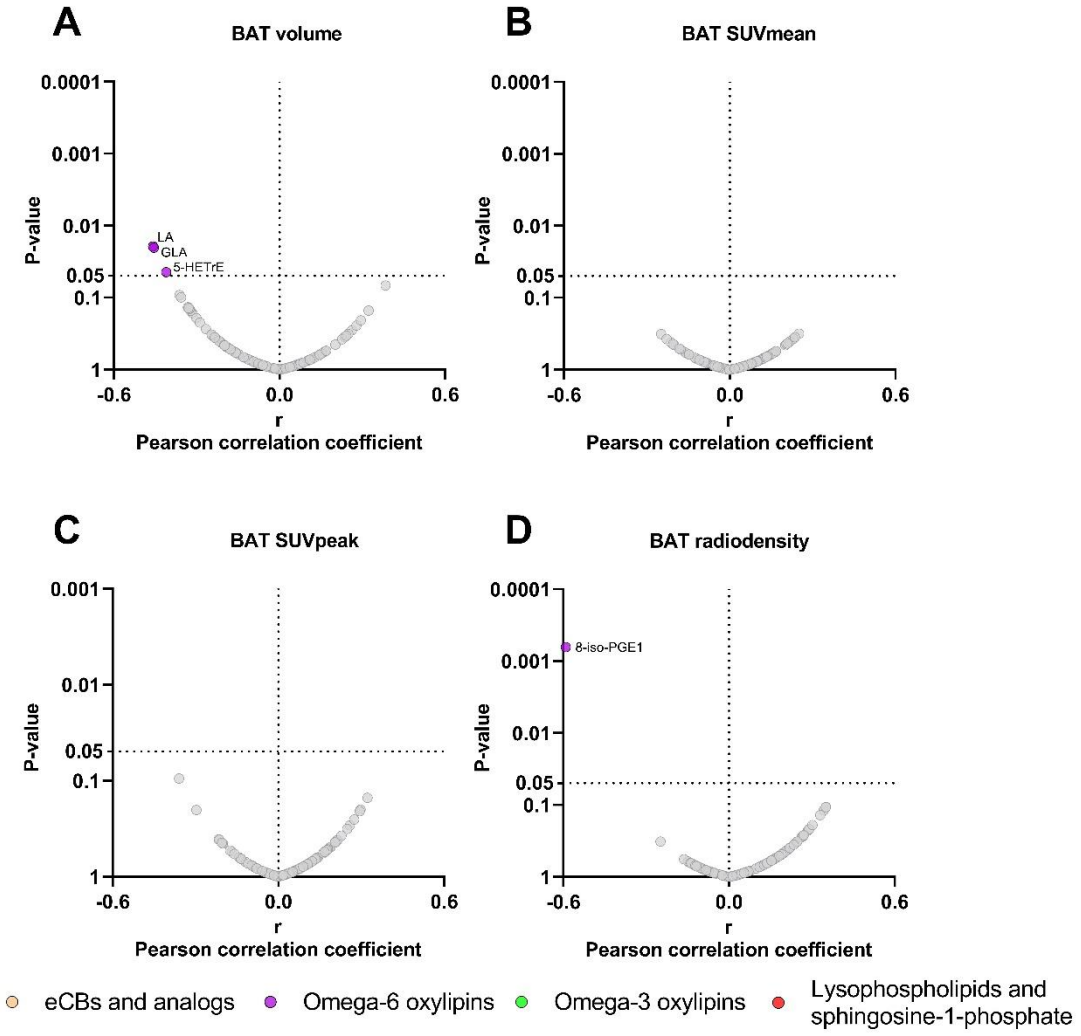

Figure S10. Association between cold-induced changes in the plasma levels of signaling lipids and brown adipose tissue-related outcomes in participants with normal-weight (n=47). Related to Figure 3.

Volcano plots showing partial correlation analyses between the 120 min fold-change relative to baseline and BAT volume (A), BAT SUVmean (B), BAT SUVpeak (C), and BAT radiodensity (D, n=33). Partial correlation analyses were adjusted for the natural calendar day when the baseline ^{18}F-FDG-PET/CT scan was performed. The X-axis represents Pearson partial correlation coefficients, whereas the Y-axis represents the FDR-adjusted P-values of the correlations. Grey dots represent non-significant correlations, whereas colored dots represent statistically significant correlations (P-value<0.05 after FDR correction). *Abbreviations*: BAT, brown adipose tissue; eCBs, endocannabinoids; SUV, standardized uptake value.

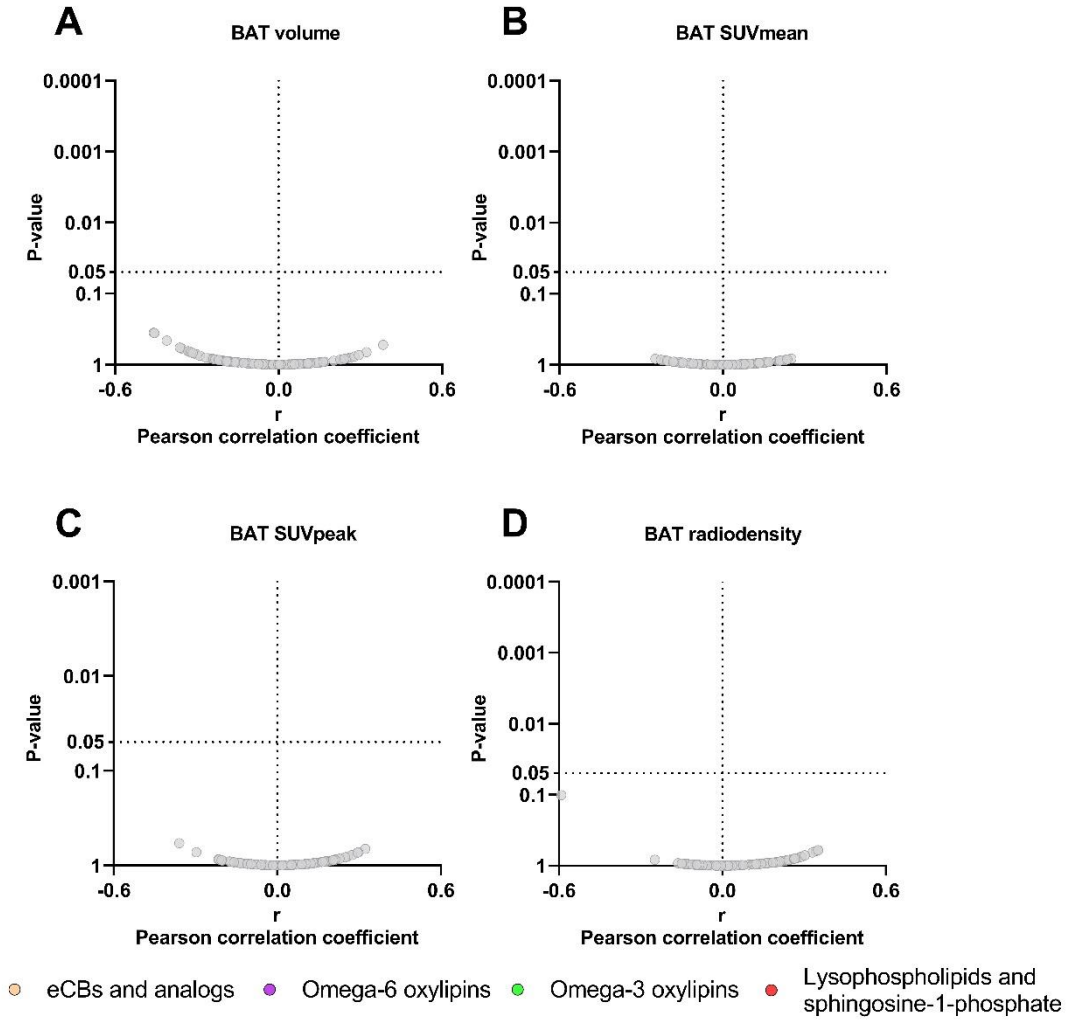

Figure S11. Association between cold-induced changes in the plasma levels of signaling lipids and brown adipose tissue-related outcomes in participants with overweight or obesity (n=21). Related to Figure 3.

Volcano plots showing partial correlation analyses between the 120 min fold-change relative to baseline and BAT volume (A), BAT SUVmean (B), BAT SUVpeak (C), and BAT radiodensity (D, n=14). Partial correlation analyses were adjusted for the natural calendar day when the baseline ^{18}F-FDG-PET/CT scan was performed. The X-axis represents Pearson partial correlation coefficients, whereas the Y-axis represents the FDR-adjusted P-values of the correlations. Grey dots represent non-significant correlations, whereas colored dots represent statistically significant correlations (P-value<0.05 after FDR correction). *Abbreviations*: BAT, brown adipose tissue; eCBs, endocannabinoids; SUV, standardized uptake value.

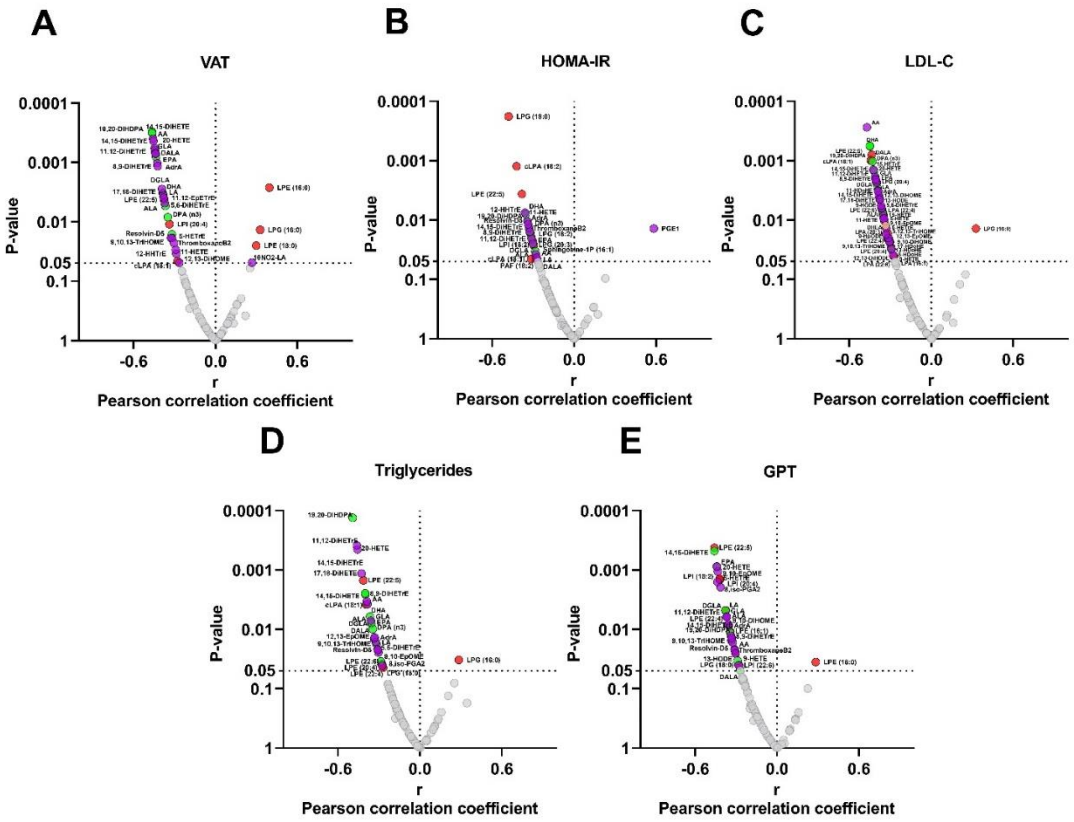

Figure S12. Association between cold-induced changes in the plasma levels of signaling lipids and cardiometabolic risk parameters. Related to Figure 4.

Volcano plots showing partial correlation analyses between the 120 min fold-change relative to baseline and VAT (A), HOMA-IR (B), LDL-C (C), triglycerides (D) and GPT (E). Partial correlation analyses were adjusted for the natural calendar day when the baseline 18F-FDG-PET/CT scan was performed. The X-axis represents Pearson partial correlation coefficients, whereas the Y-axis represents the FDR-adjusted P-values of the correlations. Grey dots represent non-significant correlations, whereas colored dots represent statistically significant correlations (P-value<0.05 after FDR correction). Abbreviations: GTP, glutamic pyruvic transaminase; HOMA-IR, homeostatic model assessment of insulin resistance index; LDL-C, low-density lipoprotein cholesterol; VAT, visceral adipose tissue.

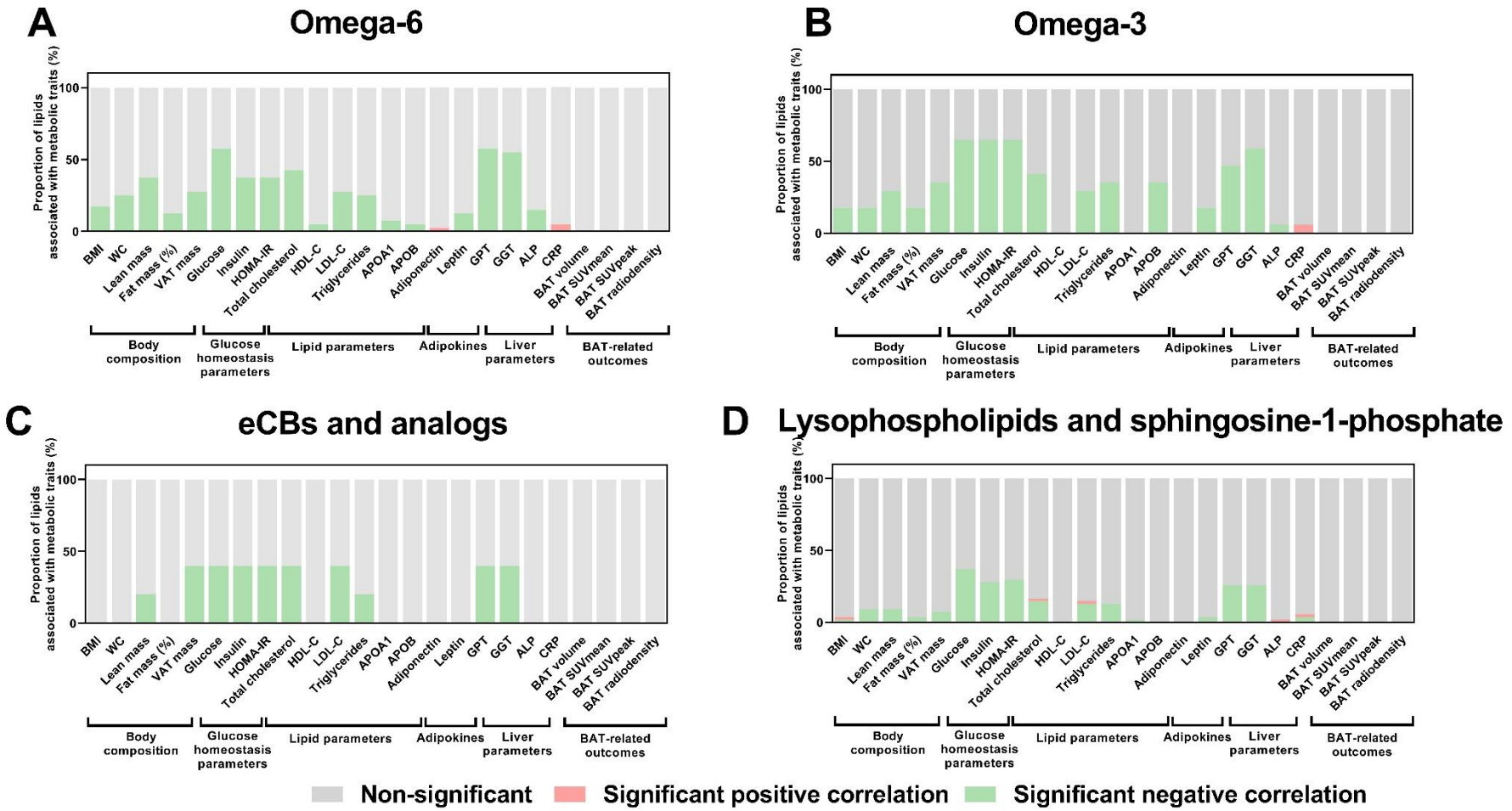

Figure S13. Relationship of cold-induced changes on the plasma levels of signaling lipids with cardiometabolic risk factors and brown adipose tissue in men (n=17). Related to Figure 4.

Bar graphs showing the proportion of cold-induced changes in omega-6 oxylipins (A), omega-3 oxylipins (B), endocannabinoids (eCBs, C), and lysophospholipids and sphingosine-1-phosphate species (D) associated with cardiometabolic risk factors. Associations were determined by Pearson bivariate correlation analyses or partial correlation analyses (for brown adipose tissue). Partial correlation analyses were adjusted for the natural calendar day when the baseline ^{18}F-FDG-PET/CT scan was performed. Cold-induced changes in plasma lipidome were determined by the 120 min fold-change relative to baseline. Significance was set at P-value<0.05 after FDR correction. Grey parts represent the proportion of lipids showing non-significant correlations, red parts represent the proportion of lipids showing significant positive correlations, whereas green parts represent the proportion of lipids showing significant negative correlations. *Abbreviations*: ALP, alkaline phosphatase; APOA1, apolipoprotein A1; APOB, apolipoprotein B; BAT, brown adipose tissue; BMI, body mass index; CRP, C-reactive protein; eCBs, endocannabinoids; GGT, gamma-glutamyl transferase; GPT, glutamic pyruvic transaminase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance index; LDL-C, low-density lipoprotein cholesterol; SUV, standardized uptake value; VAT, visceral adipose tissue; WC, waist circumference.

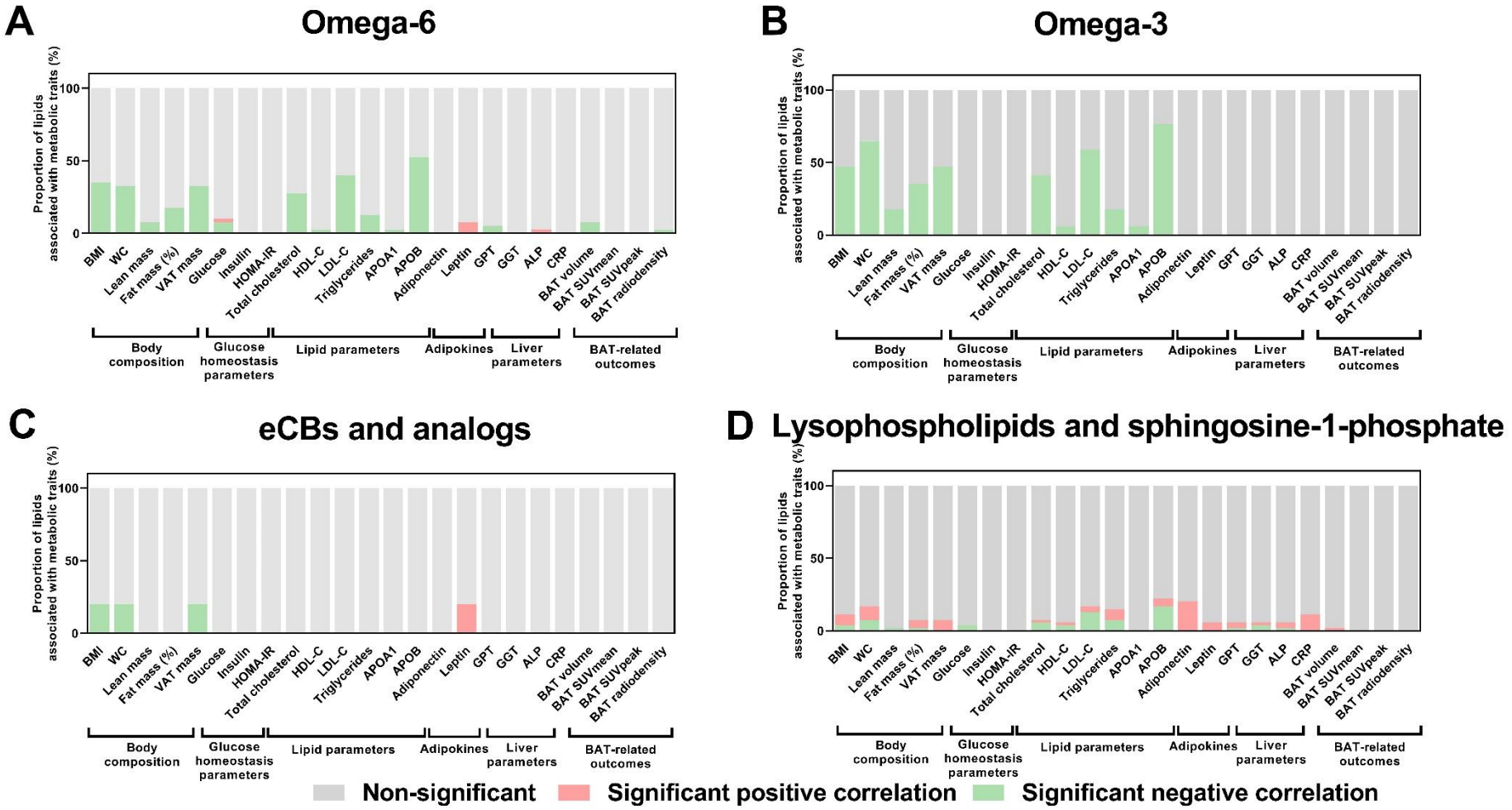

Figure S14. Relationship of cold-induced changes on the plasma levels of signaling lipids with cardiometabolic risk factors and brown adipose tissue in women (n=47). Related to Figure 4.

Bar graphs showing the proportion of cold-induced changes in omega-6 oxylipins (A), omega-3 oxylipins (B), endocannabinoids (eCBs, C), and lysophospholipids and sphingosine-1-phosphate species (D) associated with cardiometabolic risk factors. Associations were determined by Pearson bivariate correlation analyses or partial correlation analyses (for brown adipose tissue). Partial correlation analyses were adjusted for the natural calendar day when the baseline ^{18}F-FDG-PET/CT scan was performed. Cold-induced changes in plasma lipidome were determined by the 120 min fold-change relative to baseline. Significance was set at P-value<0.05 after FDR correction. Grey parts represent the proportion of lipids showing non-significant correlations, red parts represent the proportion of lipids showing significant positive correlations, whereas green parts represent the proportion of lipids showing significant negative correlations. *Abbreviations*: ALP, alkaline phosphatase; APOA1, apolipoprotein A1; APOB, apolipoprotein B; BAT, brown adipose tissue; BMI, body mass index; CRP, C-reactive protein; eCBs, endocannabinoids; GGT, gamma-glutamyl transferase; GPT, glutamic pyruvic transaminase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance index; LDL-C, low-density lipoprotein cholesterol; SUV, standardized uptake value; VAT, visceral adipose tissue; WC, waist circumference.

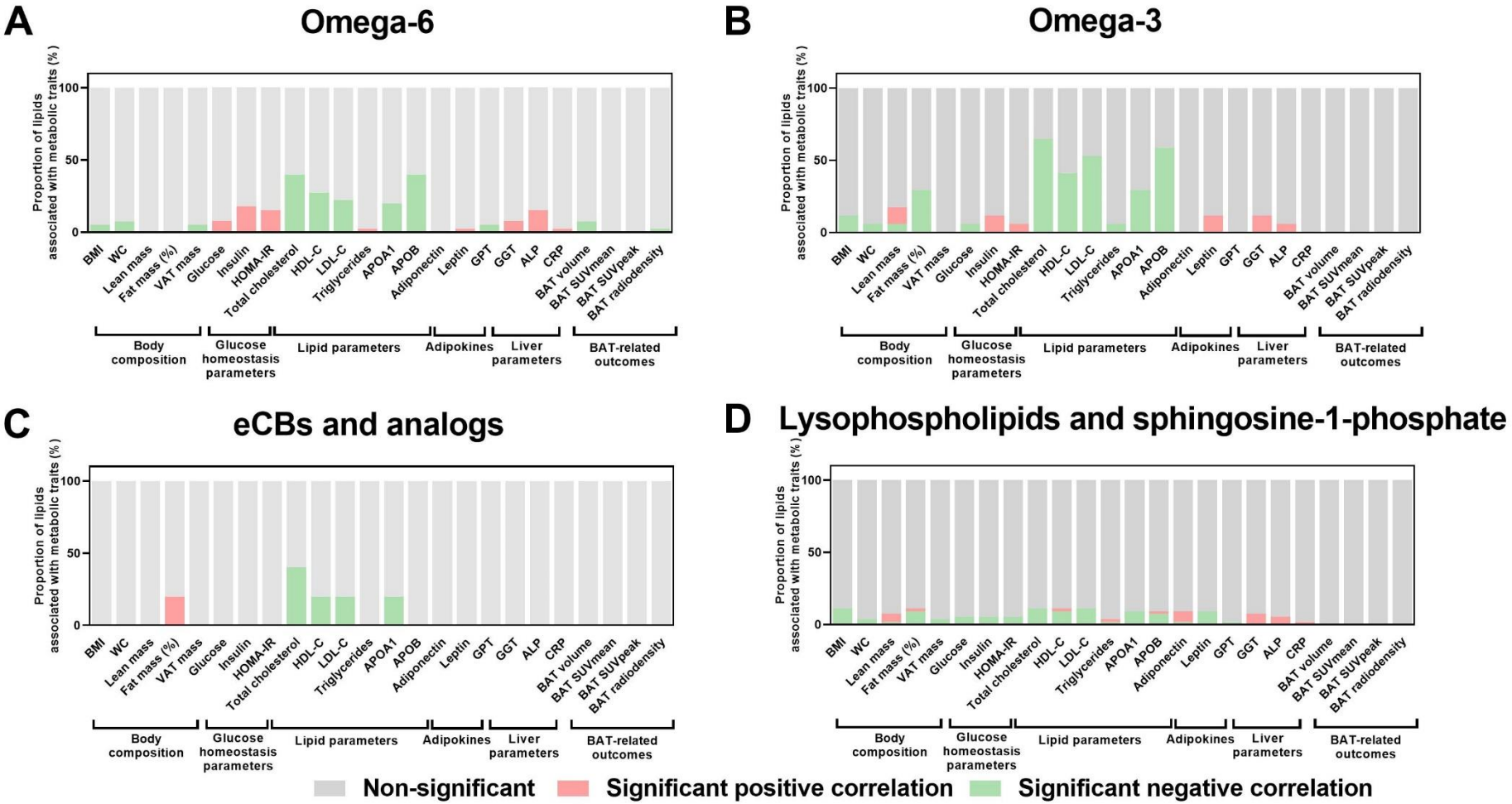

Figure S15. Relationship of cold-induced changes on the plasma levels of signaling lipids with cardiometabolic risk factors and brown adipose tissue in participants with normal-weight (n=43). Related to Figure 4.

Bar graphs showing the proportion of cold-induced changes in omega-6 oxylipins (A), omega-3 oxylipins (B), endocannabinoids (eCBs, C), and lysophospholipids and sphingosine-1-phosphate species (D) associated with cardiometabolic risk factors. Associations were determined by Pearson bivariate correlation analyses or partial correlation analyses (for brown adipose tissue). Partial correlation analyses were adjusted for the natural calendar day when the baseline ^{18}F-FDG-PET/CT scan was performed. Cold-induced changes in plasma lipidome were determined by the 120 min fold-change relative to baseline. Significance was set at P-value<0.05 after FDR correction. Grey parts represent the proportion of lipids showing non-significant correlations, red parts represent the proportion of lipids showing significant positive correlations, whereas green parts represent the proportion of lipids showing significant negative correlations. *Abbreviations*: ALP, alkaline phosphatase; APOA1, apolipoprotein A1; APOB, apolipoprotein B; BAT, brown adipose tissue; BMI, body mass index; CRP, C-reactive protein; eCBs, endocannabinoids; GGT, gamma-glutamyl transferase; GPT, glutamic pyruvic transaminase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance index; LDL-C, low-density lipoprotein cholesterol; SUV, standardized uptake value; VAT, visceral adipose tissue; WC, waist circumference.

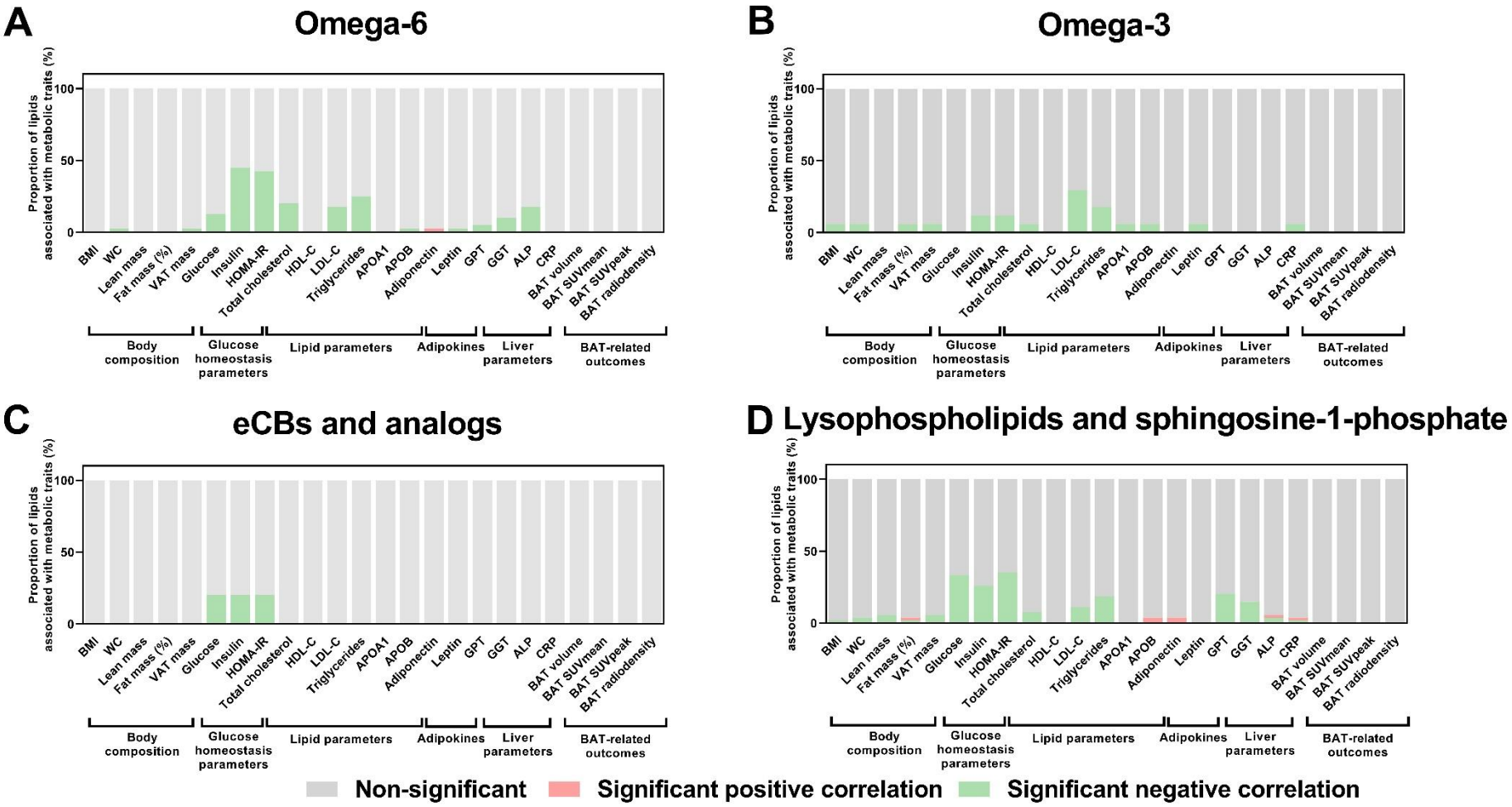

Figure S16. Relationship of cold-induced changes on the plasma levels of signaling lipids with cardiometabolic risk factors and brown adipose tissue in participants with overweight/obese (n=21). Related to Figure 4.

Bar graphs showing the proportion of cold-induced changes in omega-6 oxylipins (A), omega-3 oxylipins (B), endocannabinoids (eCBs, C), and lysophospholipids and sphingosine-1-phosphate species (D) associated with cardiometabolic risk factors. Associations were determined by Pearson bivariate correlation analyses or partial correlation analyses (for brown adipose tissue). Partial correlation analyses were adjusted for the natural calendar day when the baseline ^{18}F-FDG-PET/CT scan was performed. Cold-induced changes in plasma lipidome were determined by the 120 min fold-change relative to baseline. Significance was set at P-value<0.05 after FDR correction. Grey parts represent the proportion of lipids showing non-significant correlations, red parts represent the proportion of lipids showing significant positive correlations, whereas green parts represent the proportion of lipids showing significant negative correlations. *Abbreviations*: ALP, alkaline phosphatase; APOA1, apolipoprotein A1; APOB, apolipoprotein B; BAT, brown adipose tissue; BMI, body mass index; CRP, C-reactive protein; eCBs, endocannabinoids; GGT, gamma-glutamyl transferase; GPT, glutamic pyruvic transaminase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance index; LDL-C, low-density lipoprotein cholesterol; SUV, standardized uptake value; VAT, visceral adipose tissue; WC, waist circumference.

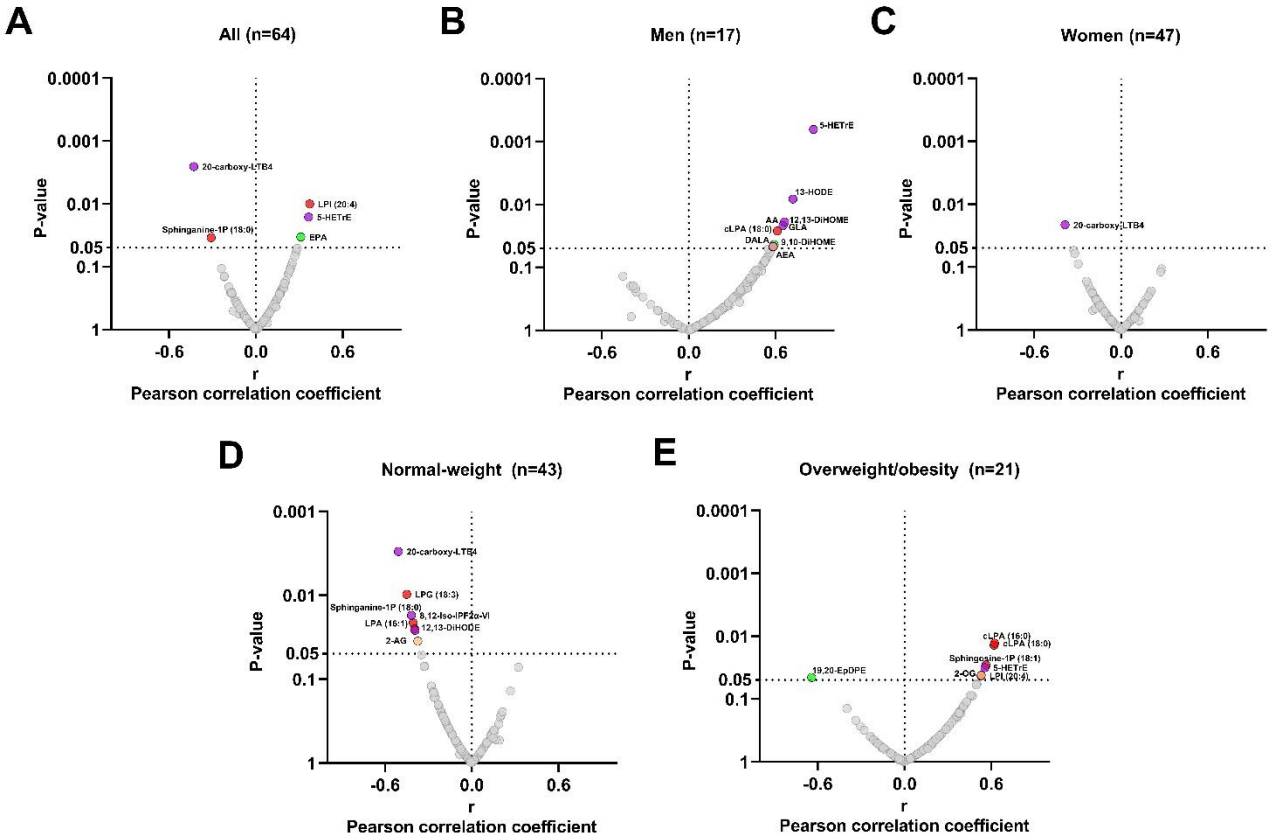

Figure S17. Association between cold-induced changes on the plasma levels of signaling lipids and the water temperature of the cooling vest. Related to Figures 3 and 4.

Volcano plots showing correlation analyses between 120 min fold-change rel. to baseline and water temperature of the cooling vest in all participants (A), men (B), women (C), normal-weight (D), and overweight/obese (E). The X-axis represents Pearson correlation coefficients, whereas the Y-axis represents the FDR-adjusted P-values of the correlations. Grey dots represent non-significant correlations, whereas colored dots represent statistically significant correlations (P-value<0.05 after FDR correction).

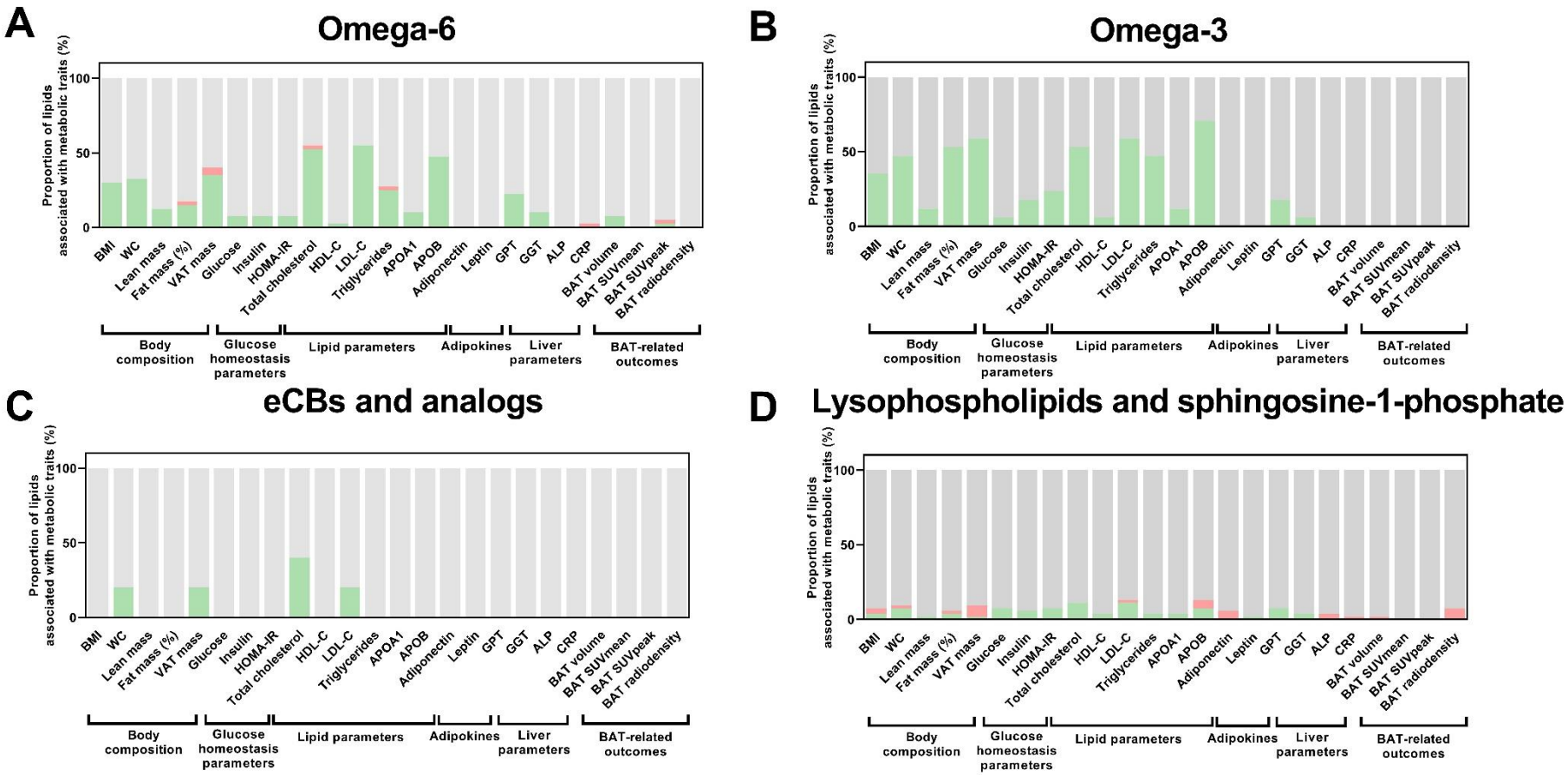

Figure S18: Relationship of cold-induced changes on the plasma levels of signaling lipids with cardiometabolic risk factors and brown adipose tissue adjusting for the water temperature of the cooling vest. Related to Figure 4.

Bar graphs showing the proportion of cold-induced changes in omega-6 oxylipins (A), omega-3 oxylipins (B), endocannabinoids (eCBs, C), and lysophospholipids and sphingosine-1-phosphate species (D) associated with cardiometabolic risk factors. Associations determined by Pearson partial correlation analyses adjusted for the water temperature of the cooling vest and for both the water temperature of the cooling vest and the natural calendar day when the baseline ^{18}F-FDG-PET/CT scan was performed (for brown adipose tissue). Cold-induced changes in plasma lipidome were determined by the 120 min fold-change relative to baseline. Significance was set at P-value<0.05 after FDR correction. Grey parts represent the proportion of lipids showing non-significant correlations, red parts represent the proportion of lipids showing significant positive correlations, whereas green parts represent the proportion of lipids showing significant negative correlations. *Abbreviations*: ALP, alkaline phosphatase; APOA1, apolipoprotein A1; APOB, apolipoprotein B; BAT, brown adipose tissue; BMI, body mass index; CRP, C-reactive protein; eCBs, endocannabinoids; GGT, gamma-glutamyl transferase; GPT, glutamic pyruvic transaminase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance index; LDL-C, low-density lipoprotein cholesterol; SUV, standardized uptake value; VAT, visceral adipose tissue; WC, waist circumference.