

# Supplementary Material

## Technical Report: A Comprehensive Comparison between Different Quantification Versions of Nightingale Health's <sup>1</sup>H-NMR Metabolomics Platform

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<sup>†</sup> BBMRI-NL: <https://www.bbmri.nl>; see Consortium Banner Supplement S1.

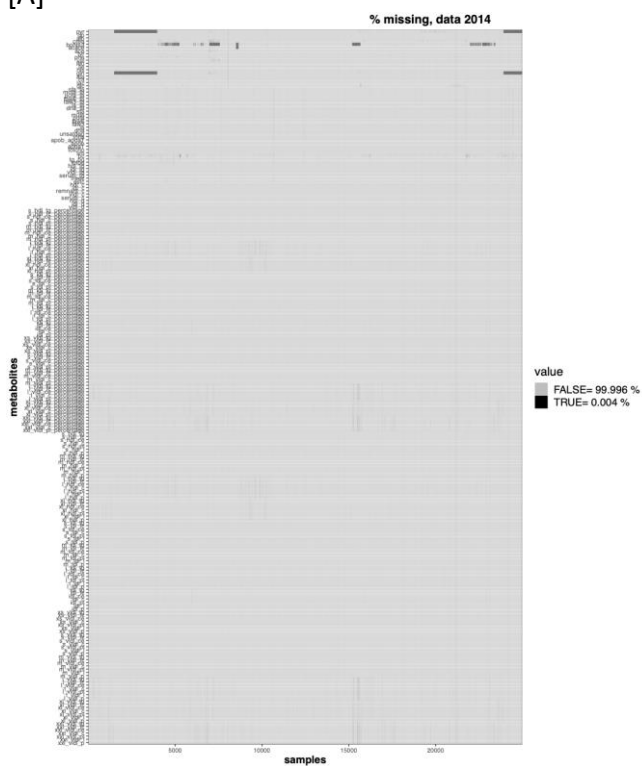
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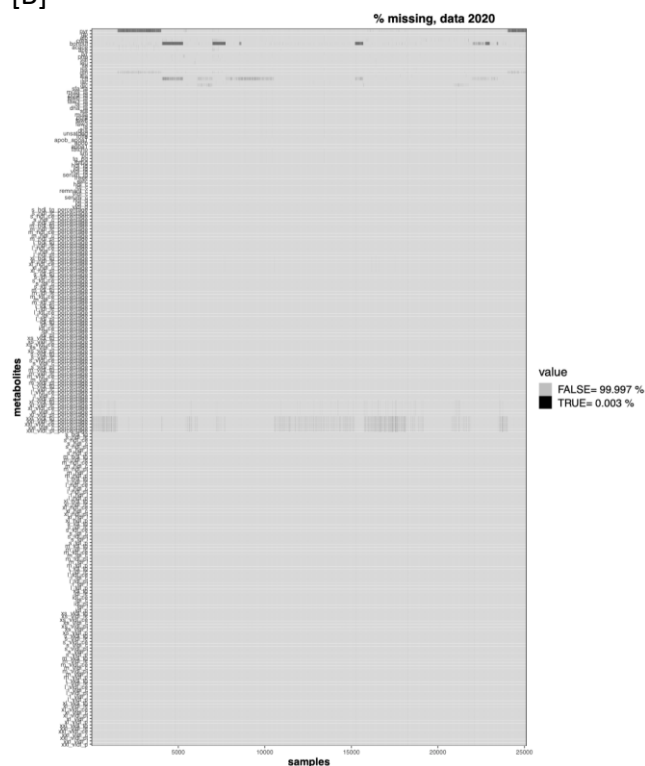
Supplementary Figures

Figure S1

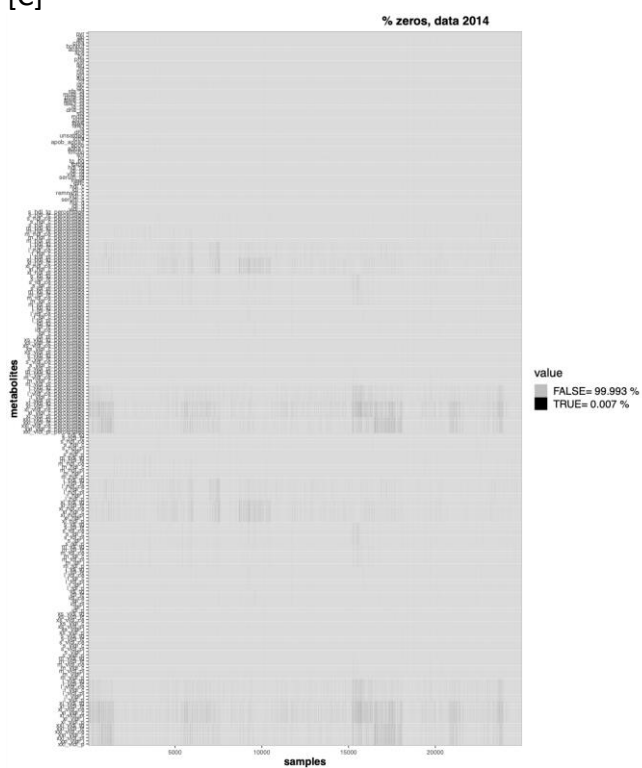
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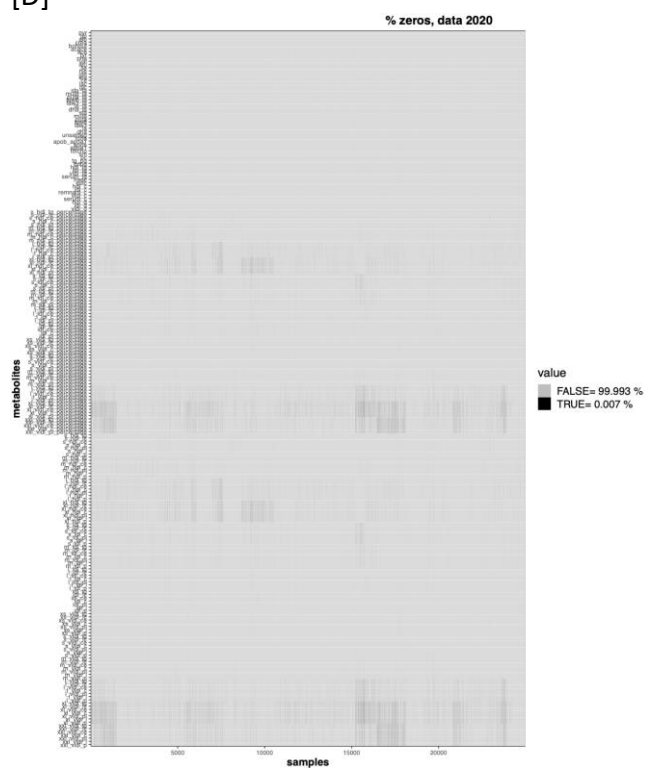
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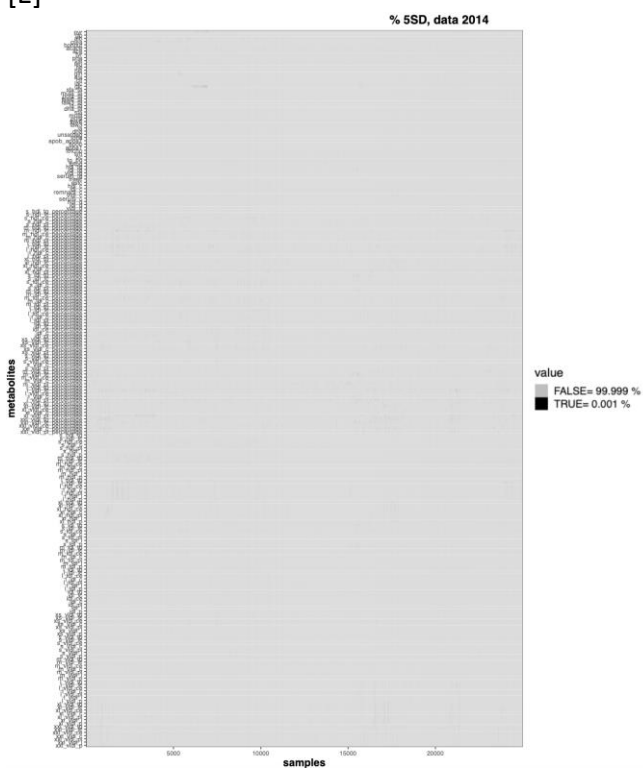
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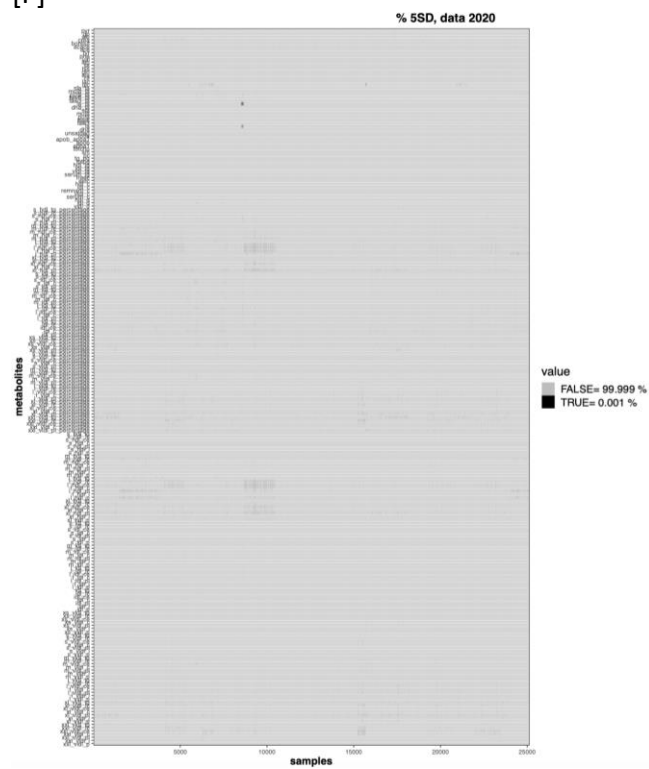
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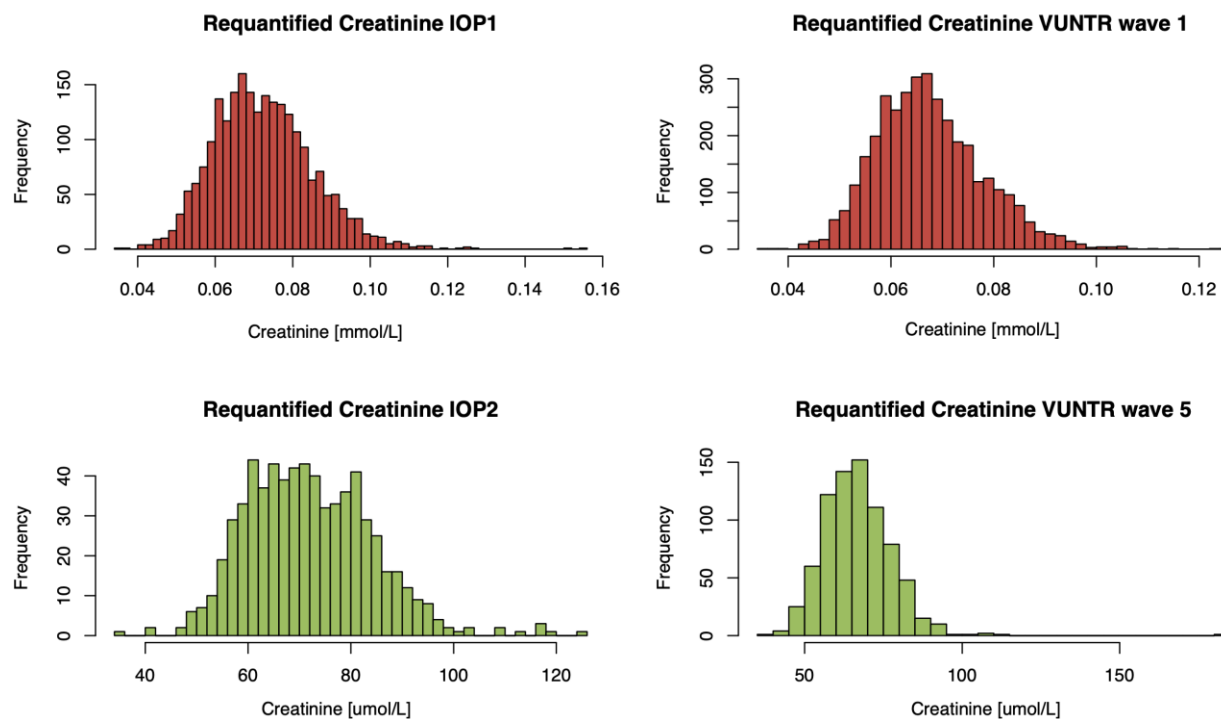


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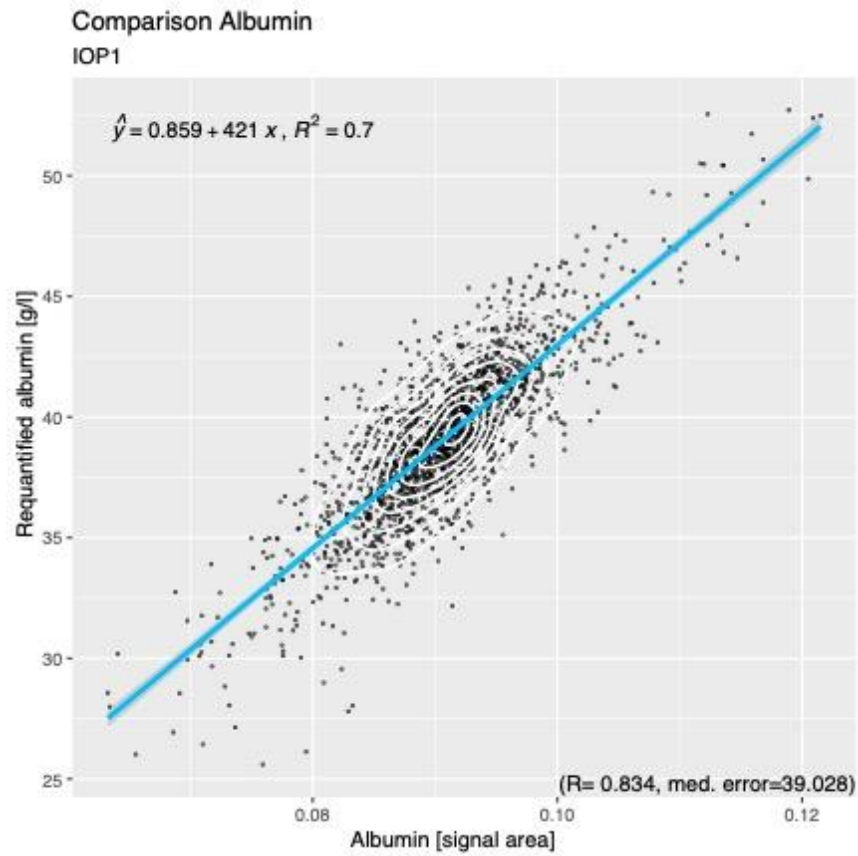
**Figure S1:** Heatmaps reporting in black the samples with [A-B] missing, [C-D] zeros, and [E-F] outliers in the Nightingale Health metabolomics dataset of 2014 and 2020.

Figure S2



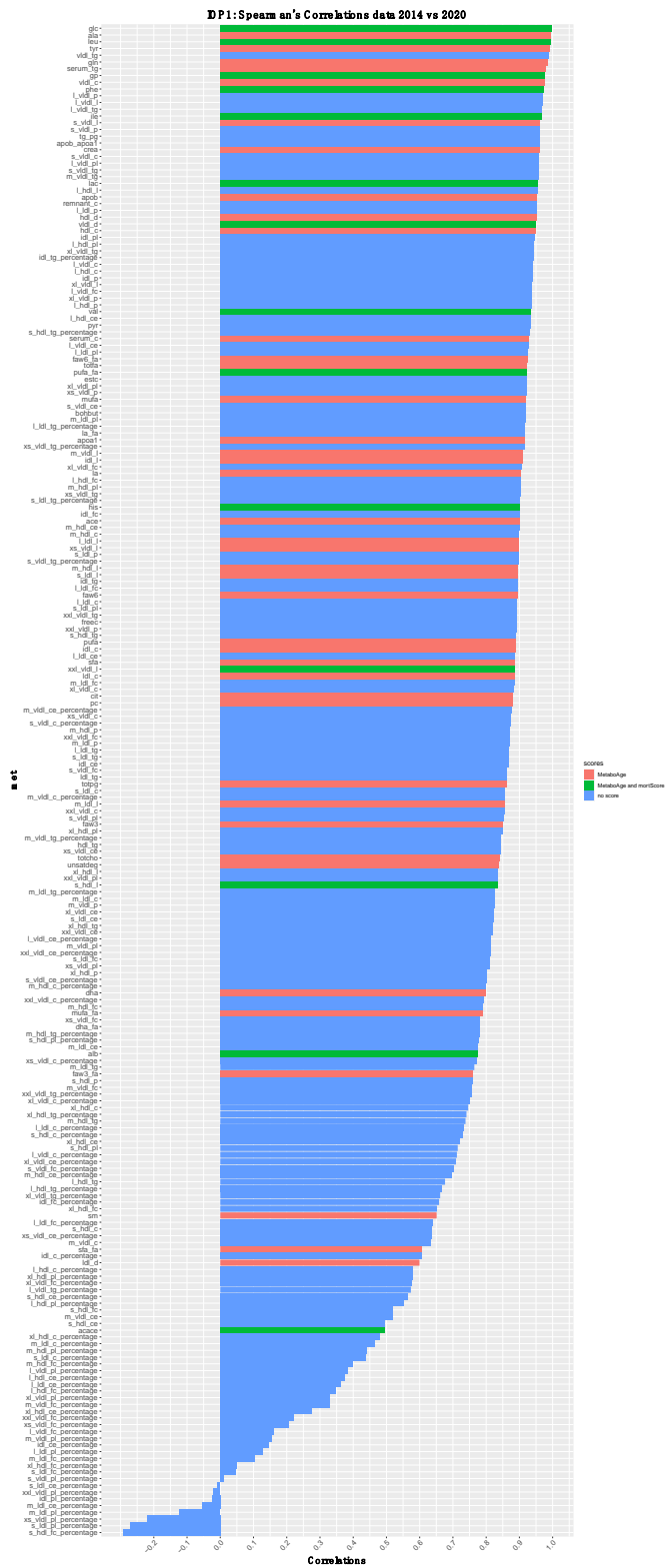
**Figure S2:** Distribution of creatinine in the Nightingale Metabolomics dataset of 2020. On the left the distributions in LLS-PAROFFS IOP1 (top), which was quantified the first time in 2014 and the repeated measures of IOP2 (bottom), quantified the first time in 2016. On the right wave 1 (top), quantified the first time in 2014, and wave 5 (bottom), quantified the first time in 2016, of VUNTR.

Figure S3



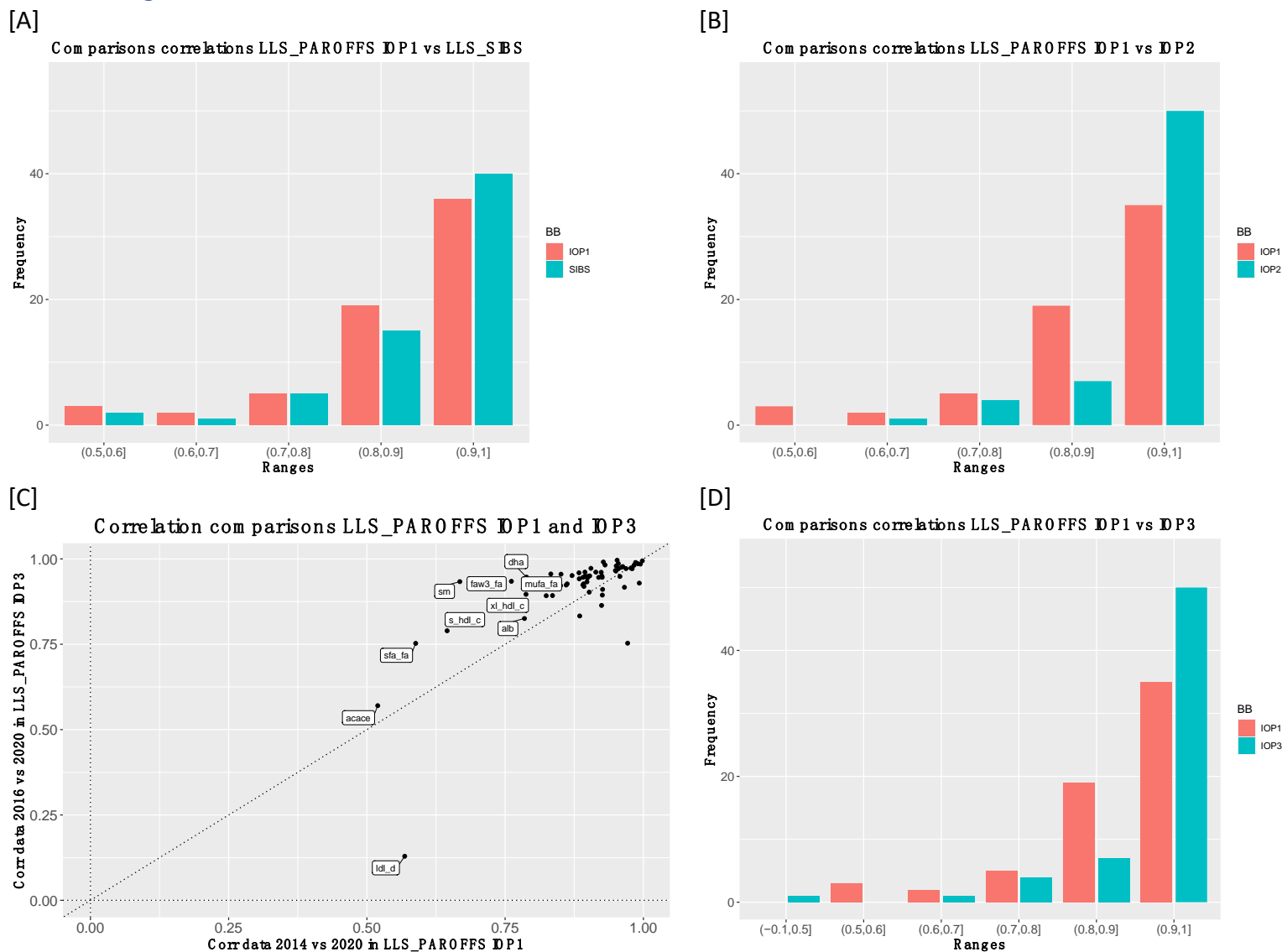
**Figure S3:** Comparison Albumin measured in the first wave (x-axis) and re-quantified (y-axis), with axes adjusted to the ranges of each variable.

Figure S4



**Figure S4:** Spearman's correlations comparing the 2014 and 2020 versions of all 220 metabolomics features.

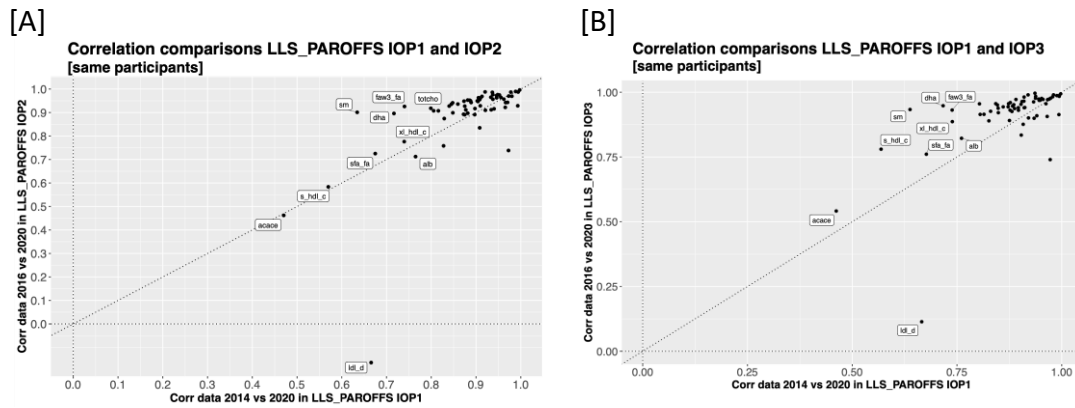
Figure S5



**Figure S5:** Comparisons of the correlations of the metabolites before and after re-quantification in LLS-SIBS and LLS-PAROFFS IOP1,2 and 3: [A] Bar-plot comparing of the correlations of the metabolites in IOP1 and LLS-SIBS divided in ranges, [B] Bar-plot comparing of the correlations in LLS-PAROFFS IOP1 and 2, [C] Scatterplot comparing the correlations of LLS-PAROFFS IOP1 and IOP3 and [D] Boxplot of the correlations in LLS-PAROFFS IOP1 and IOP3 divided in ranges.



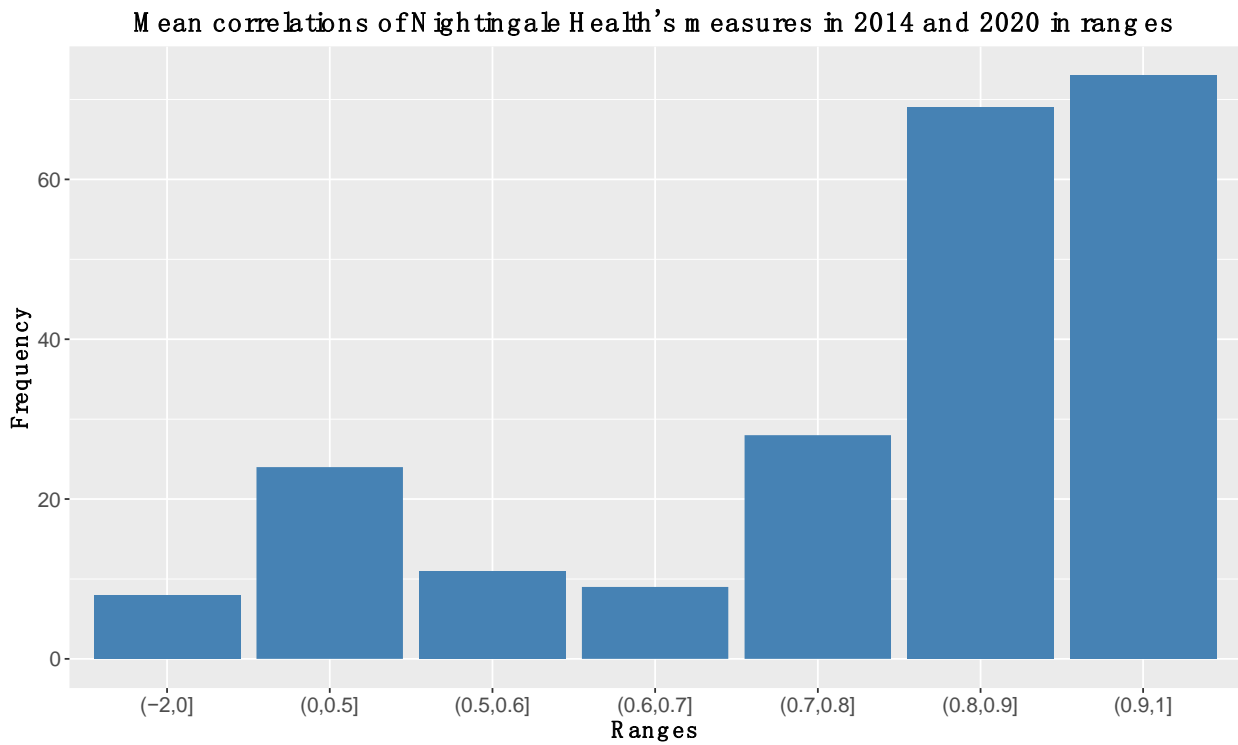
Figure S6



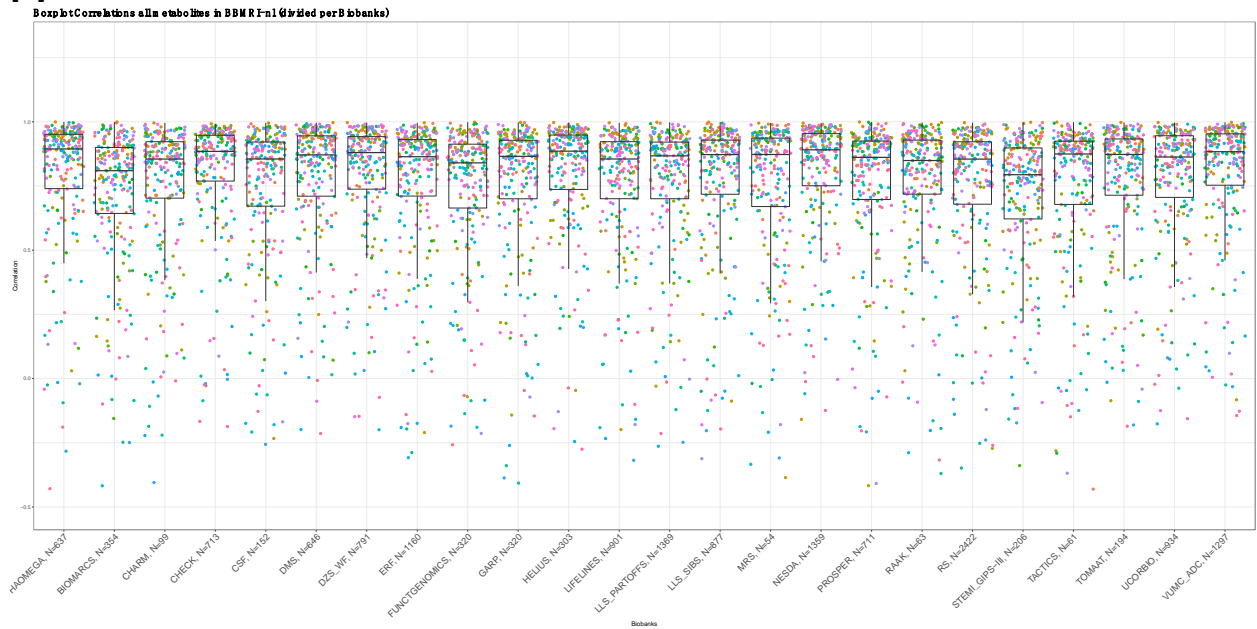
**Figure S6.** Comparisons of the Spearman's correlations of each metabolic markers before and after the re-quantifications in a subset of LLS\_PAROFFS composed by the samples of the same 306 study participants that participated to all three measurement (IOP1, IOP2, and IOP3). [A] compares the correlations of LLS-PAROFFS IOP1 and IOP2 and [B] compares the Spearman's correlations in IOP1 and IOP

Figure S7

[A]



[B]



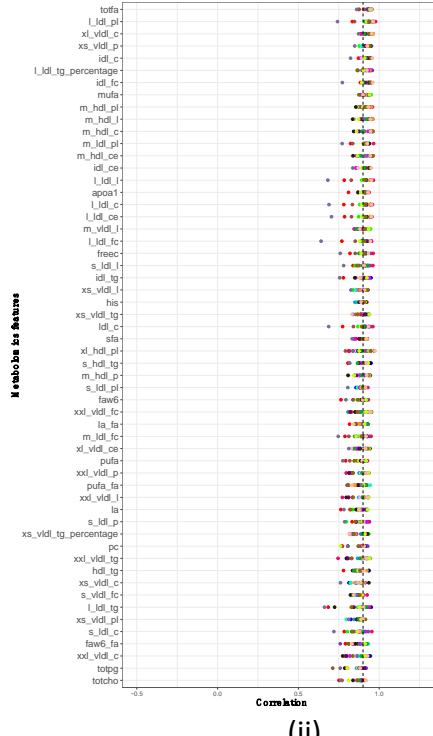
[C]

Correlations of the metabolic features in the BBMRI-nicohorts  
[1st quartile]



(i)

Correlations of the metabolic features in the BBMRI-nicohorts  
[2nd quartile]



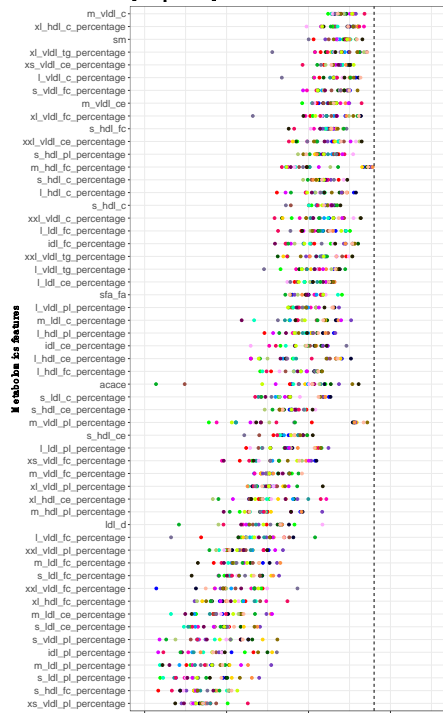
(ii)

Correlations of the metabolic features in the BBMRI-nicohorts  
[3rd quartile]



(iii)

Correlations of the metabolic features in the BBMRI-nicohorts  
[4th quartile]



(iv)

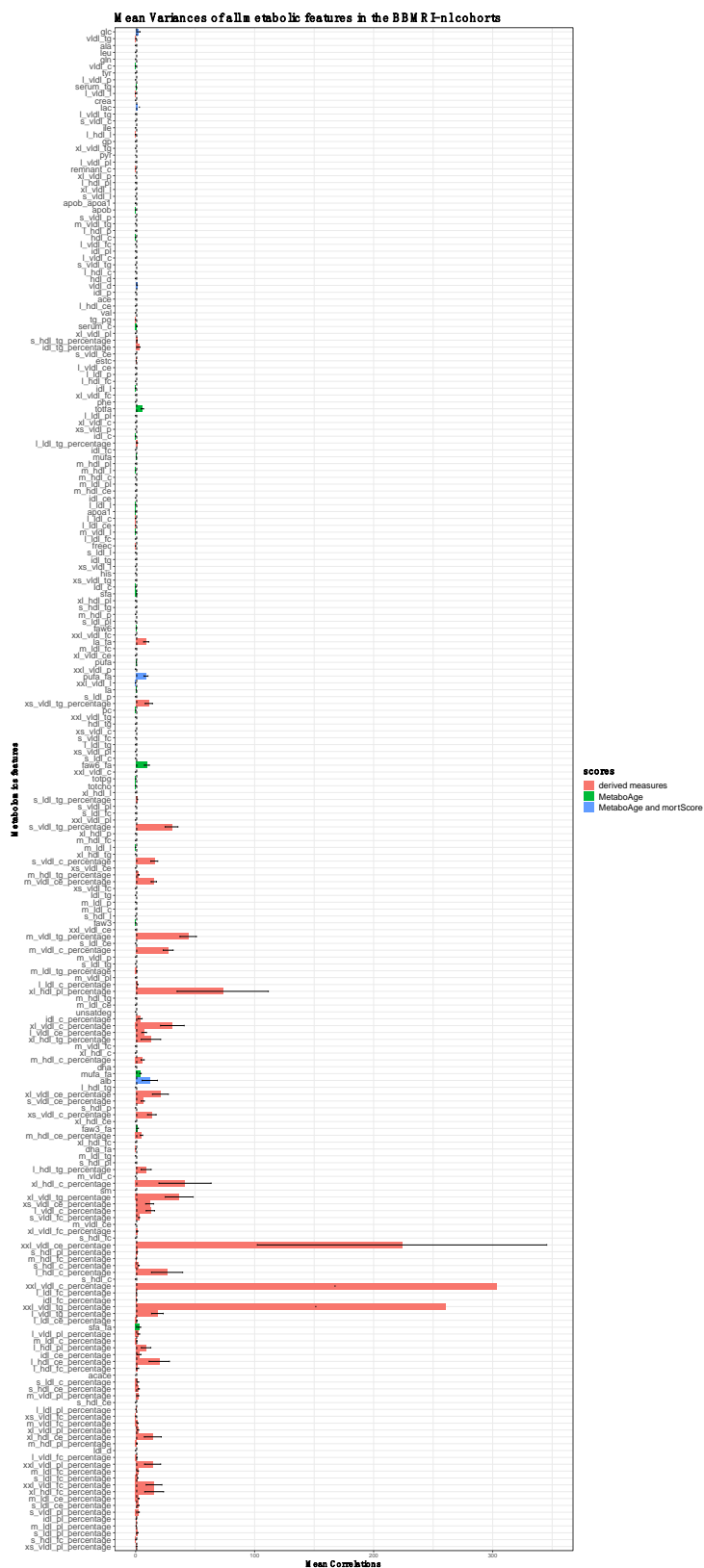
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  - CHARM, N=99
  - CHECK, N=713
  - CSF, N=152
  - DMS, N=646
  - DZS\_WF, N=791
  - ERF, N=1159
  - FUNCTGENOMICS, N=317
  - GARP, N=320
  - HELIUS, N=303
  - LIFELINES, N=900
  - LLS\_PARTOFFS, N=1369
  - LLS\_SIBS, N=877
  - MRS, N=54
  - NESDA, N=1359
  - PROSPER, N=712
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  - RS, N=2422
  - STEMI\_GIPS-III, N=206
  - TACTICS, N=61
  - TOMAAT, N=194
  - UCORBIO, N=934
  - VUMC\_ADC, N=1297

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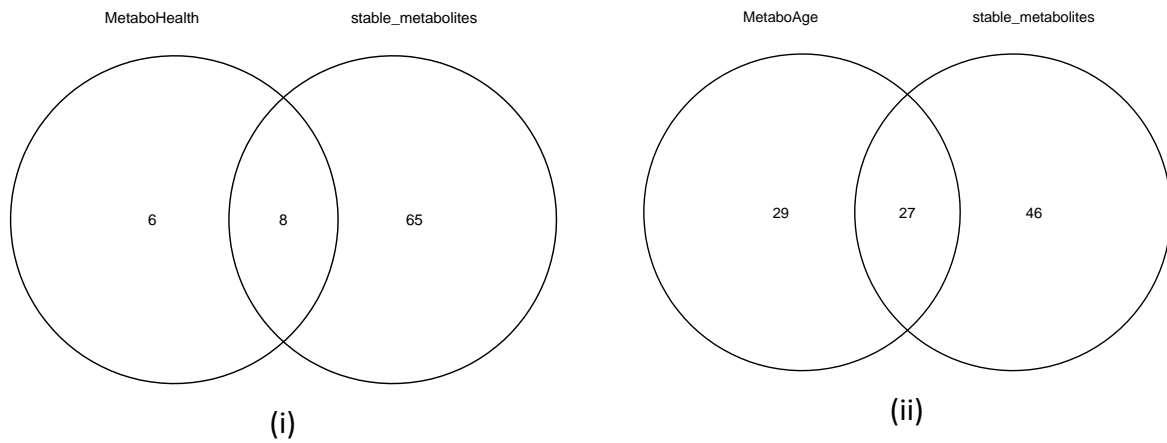
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[D]



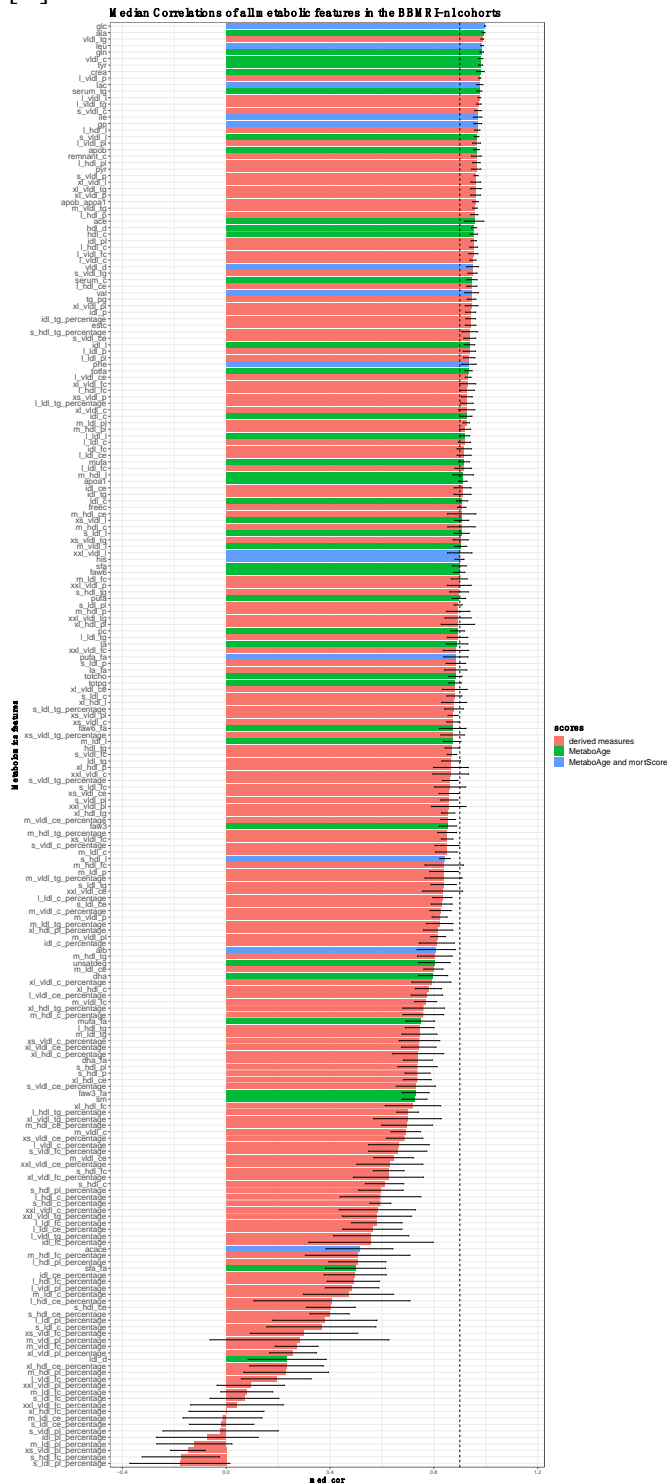
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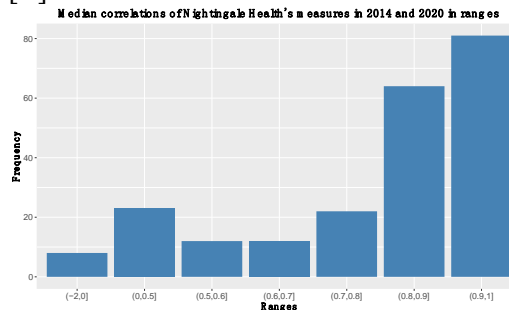
**Figure S7:** Spearman's correlations of all 220 metabolomics analytes in all the biobanks of BBMRI-nl before (2014) and after the re-quantifications (2020). [A] Histogram of the quantities of features divided in ranges of correlation values. Dot plot representing the correlations split in biobanks [B] and metabolic features [C]. The dots are colored based on the cohort. In [C] the dots of the same cohort are connected by a line with the same color, moreover the features are split in quartiles of their correlations to allow for a better visualization. [D] variance of the 2020 versions of all the features in all the biobanks in BBMRI-nl, [E] Venn diagrams representing the overlap of the stable metabolites with (i) MetaboHealth and (ii) MetaboAge.

Figure S8

[A]



[B]



**Figure S8:** Medians of the Spearman's correlations of all 220 metabolomics analytes in all analytes in all the biobanks of BBMRI-nl before (2014) and after the re-quantifications (2020). [A] Bar plot of the median and MAD of the Spearman's correlation for each metabolomics feature, [B] Histogram of the quantities of features divided in ranges of median correlation values.

Figure S9

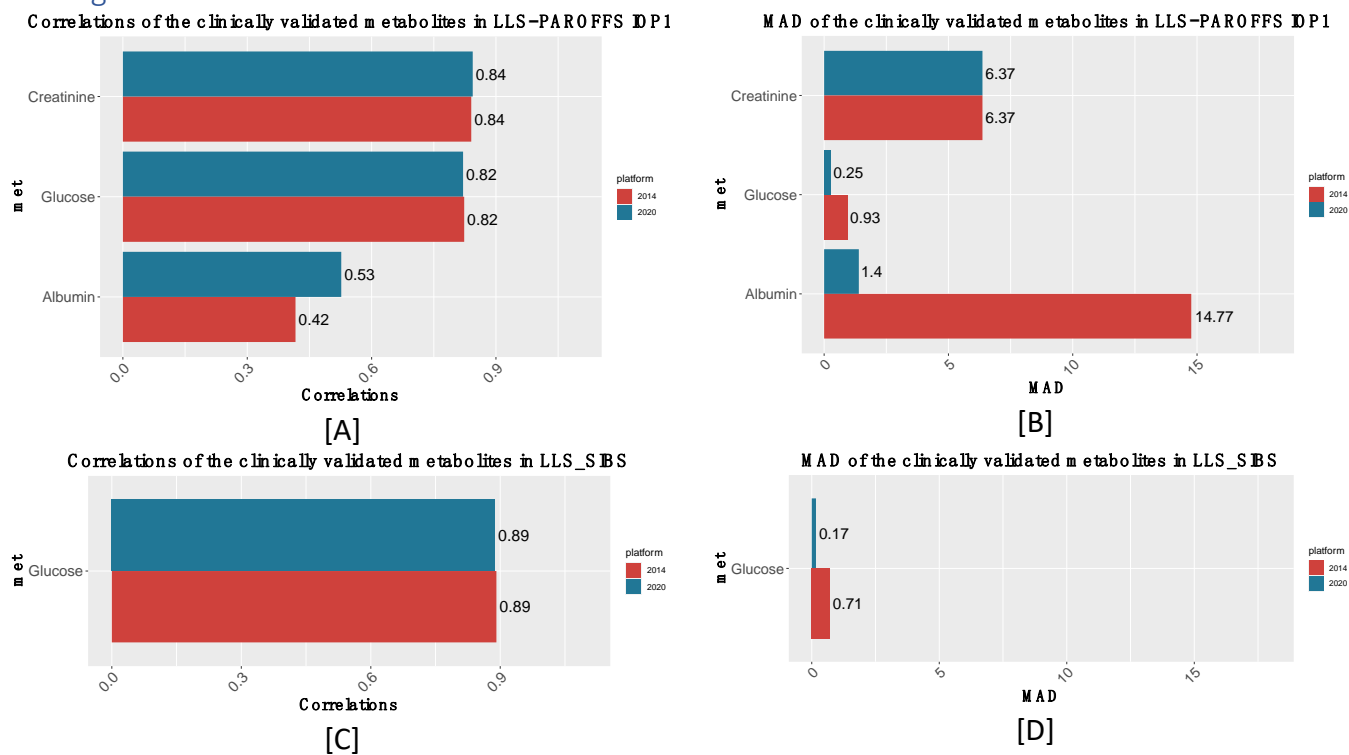
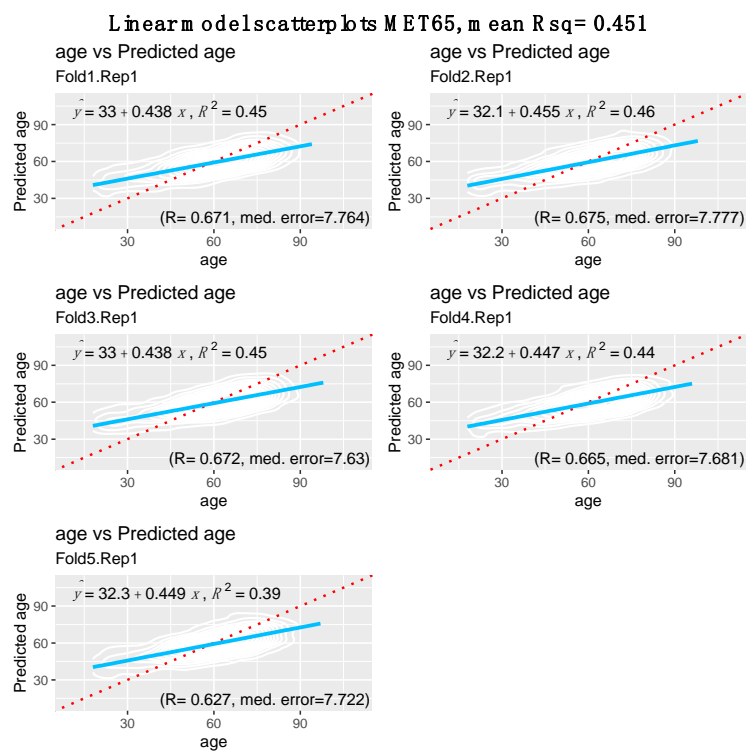


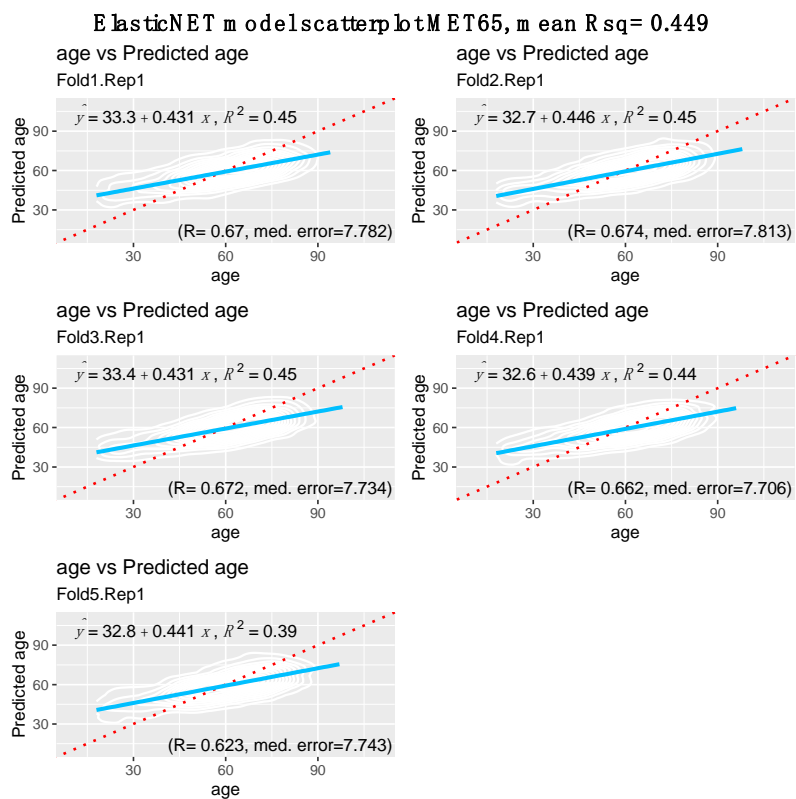
Figure S9: Comparisons of Nightingale metabolomics markers, measured in 2014 (red) and 2020 (blue), with the clinically measured values in the Leiden Longevity Study: Bar-plots of the (A-C) Spearman's correlations and (B-D) the Median Absolute Distance (MAD).

Figure S10

[A]



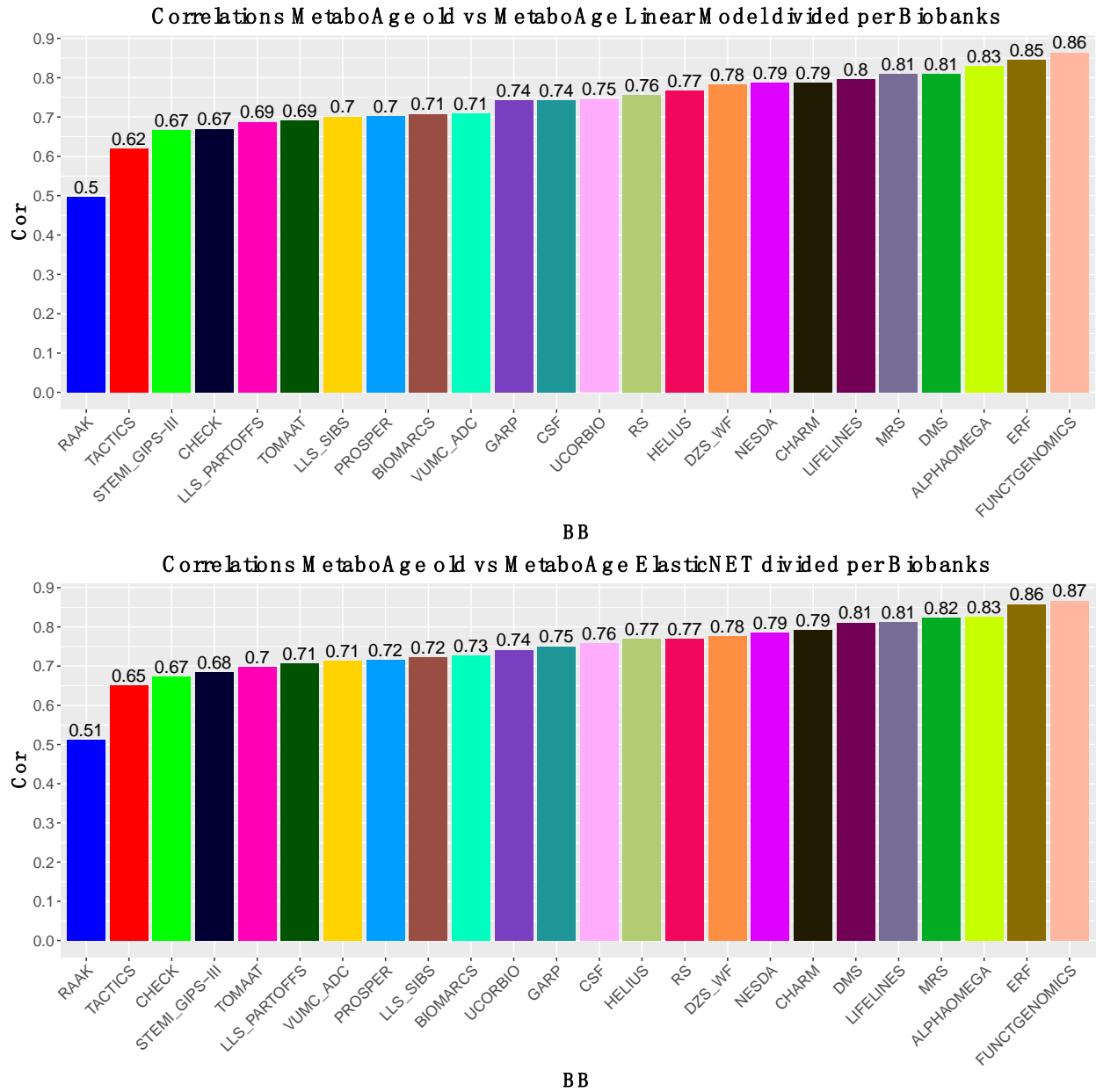
[B]





**Figure S10:** 5-Fold Cross-Validation results for the MetaboAge 2.0 for [A] the linear model and [B] the ElasticNET model.

Figure S11



**Figure S11:** Correlations of MetaboAge 1.0 and MetaboAge 2.0 ([A] linear model and [B] ElasticNET) across all the cohorts in BBMRI-nl.

## Supplementary tables

Table S1: List of the 65 metabolites used to build the novel MetaboAge

BBMRI_names	description
serum_c	Total cholesterol
vldl_c	VLDL cholesterol
ldl_c	LDL cholesterol
hdl_c	HDL cholesterol
serum_tg	Total triglycerides
vldl_d	Average diameter for VLDL particles
ldl_d	Average diameter for LDL particles
hdl_d	Average diameter for HDL particles
totpg	Phosphoglycerides
totcho	Total cholines
pc	Phosphatidylcholines
sm	Sphingomyelins
apob	Apolipoprotein B
apoa1	Apolipoprotein A1
totfa	Total fatty acids
unsatdeg	Degree of unsaturation
faw3	Omega-3 fatty acids
faw6	Omega-6 fatty acids
pufa	Polyunsaturated fatty acids
mufa	Monounsaturated fatty acids
sfa	Saturated fatty acids
la	Linoleic acid
dha	Docosahexaenoic acid
faw3_fa	Ratio of omega-3 fatty acids to total fatty acids
faw6_fa	Ratio of omega-6 fatty acids to total fatty acids
pufa_fa	Ratio of polyunsaturated fatty acids to total fatty acids
mufa_fa	Ratio of monounsaturated fatty acids to total fatty acids
sfa_fa	Ratio of saturated fatty acids to total fatty acids
ala	Alanine
gln	Glutamine
his	Histidine
ile	Isoleucine
leu	Leucine
val	Valine
phe	Phenylalanine

tyr	Tyrosine
glc	Glucose
lac	Lactate
pyr	Pyruvate
cit	Citrate
bohbut	3-Hydroxybutyrate
ace	Acetate
acace	Acetoacetate
crea	Creatinine
alb	Albumin
gp	Glycoprotein acetyls
xxl_vldl_l	Total lipids in chylomicrons and extremely large VLDL
xl_vldl_l	Total lipids in very large VLDL
l_vldl_l	Total lipids in large VLDL
m_vldl_l	Total lipids in medium VLDL
s_vldl_l	Total lipids in small VLDL
xs_vldl_l	Total lipids in very small VLDL
idl_l	Total lipids in IDL
idl_c	Cholesterol in IDL
l_ldl_l	Total lipids in large LDL
m_ldl_l	Total lipids in medium LDL
s_ldl_l	Total lipids in small LDL
xl_hdl_l	Total lipids in very large HDL
xl_hdl_c	Cholesterol in very large HDL
l_hdl_l	Total lipids in large HDL
l_hdl_c	Cholesterol in large HDL
m_hdl_l	Total lipids in medium HDL
m_hdl_c	Cholesterol in medium HDL
s_hdl_l	Total lipids in small HDL
s_hdl_c	Cholesterol in small HDL

Table S2: Discontinued metabolites in the platform 2020

BBMRI_names	description
hdl2_c	HDL2 cholesterol
hdl3_c	HDL3 cholesterol

Table S3: Metabolites added to the Nightingale platform with the update of 2020

BBMRI_names	description
-------------	-------------

non_hdl_c	Total cholesterol minus HDL-C
clinical_ldl_c	Clinical LDL cholesterol
total_pl	Total phospholipids in lipoprotein particles
hdl_pl	Phospholipids in HDL
ldl_pl	Phospholipids in LDL
vldl_pl	Phospholipids in VLDL
hdl_ce	Cholesteryl esters in HDL
ldl_ce	Cholesteryl esters in LDL
vldl_ce	Cholesteryl esters in VLDL
hdl_fc	Free cholesterol in HDL
ldl_fc	Free cholesterol in LDL
vldl_fc	Free cholesterol in VLDL
total_l	Total lipids in lipoprotein particles
hdl_l	Total lipids in HDL
ldl_l	Total lipids in LDL
vldl_l	Total lipids in VLDL
total_p	Total concentration of lipoprotein particles
hdl_p	Concentration of HDL particles
ldl_p	Concentration of LDL particles
vldl_p	Concentration of VLDL particles
pufa_by_mufo	Ratio of polyunsaturated fatty acids to monounsaturated fatty acids
faw6_by_faw3	Ratio of omega-6 fatty acids to omega-3 fatty acids
total_bcaa	Total concentration of branched-chain amino acids (leucine + isoleucine + valine)
acetone	Acetone

Table S4: Clinically validated metabolites

BBMRI_names	description
serum_c	Total cholesterol
vldl_c	VLDL cholesterol
clinical_ldl_c	Clinical LDL cholesterol
hdl_c	HDL cholesterol
serum_tg	Total triglycerides
apob	Apolipoprotein B
apoal	Apolipoprotein A1
apob_apoal	Ratio of apolipoprotein B to apolipoprotein A1
totfa	Total fatty acids
faw3	Omega-3 fatty acids
faw6	Omega-6 fatty acids
pufo	Polyunsaturated fatty acids

mufa	Monounsaturated fatty acids
sfa	Saturated fatty acids
dha	Docosahexaenoic acid
faw3_fa	Ratio of omega-3 fatty acids to total fatty acids
faw6_fa	Ratio of omega-6 fatty acids to total fatty acids
pufa_fa	Ratio of polyunsaturated fatty acids to total fatty acids
mufa_fa	Ratio of monounsaturated fatty acids to total fatty acids
sfa_fa	Ratio of saturated fatty acids to total fatty acids
dha_fa	Ratio of docosahexaenoic acid to total fatty acids
pufa_by_mufa	Ratio of polyunsaturated fatty acids to monounsaturated fatty acids
faw6_by_faw3	Ratio of omega-6 fatty acids to omega-3 fatty acids
ala	Alanine
glycine	Glycine
his	Histidine
total_bcaa	Total concentration of branched-chain amino acids (leucine + isoleucine + valine)
ile	Isoleucine
leu	Leucine
val	Valine
phe	Phenylalanine
tyr	Tyrosine
glc	Glucose
lac	Lactate
crea	Creatinine
alb	Albumin

Table S5: 73 stable metabolites

BBMRI metabolites name	Description	Mean correlations	SD correlations	Median correlations	MAD correlations	Presence in scores
l_ldl_ce	Cholesteryl esters in large LDL	0.9028	0.0571	0.9163	0.0268	derived measures
l_ldl_c	Cholesterol in large LDL	0.903	0.0597	0.9176	0.0234	derived measures
apoa1	Apolipoprotein A1	0.9042	0.0275	0.9114	0.0161	MetaboAge
l_ldl_l	Total lipids in large LDL	0.9048	0.0613	0.9183	0.0194	MetaboAge
idl_ce	Cholesteryl esters in IDL	0.9078	0.0315	0.9105	0.0327	derived measures
m_hdl_ce	Cholesteryl esters in medium HDL	0.9103	0.0398	0.9071	0.0543	derived measures
m_ldl_pl	Phospholipids in medium LDL	0.9106	0.0421	0.9248	0.0123	derived measures
m_hdl_c	Cholesterol in medium HDL	0.9119	0.0381	0.9063	0.0527	derived measures
m_hdl_l	Total lipids in medium HDL	0.9136	0.0352	0.9126	0.0397	MetaboAge
m_hdl_pl	Phospholipids in medium HDL	0.9148	0.0268	0.92	0.0214	derived measures
mufa	Monounsaturated fatty acids	0.9151	0.0184	0.9161	0.0211	MetaboAge

idl_fc	Free cholesterol in IDL	0.9153	0.0376	0.9167	0.0274	derived measures
l_ldl_tg_percentage	Triglycerides to total lipids ratio in large LDL	0.9194	0.0266	0.927	0.0239	derived measures
idl_c	Cholesterol in IDL	0.9198	0.0299	0.925	0.0217	MetaboAge
xs_vldl_p	Concentration of very small VLDL particles	0.9207	0.0243	0.9272	0.0207	derived measures
xl_vldl_c	Cholesterol in very large VLDL	0.9236	0.0268	0.926	0.0311	derived measures
l_ldl_pl	Phospholipids in large LDL	0.9244	0.051	0.936	0.0221	derived measures
totfa	Total fatty acids	0.9272	0.0255	0.9335	0.0139	MetaboAge
phe	Phenylalanine	0.9276	0.0523	0.935	0.0277	MetaboAge and mortScore
xl_vldl_fc	Free cholesterol in very large VLDL	0.9288	0.0263	0.9303	0.0308	derived measures
idl_l	Total lipids in IDL	0.9291	0.0331	0.937	0.02	MetaboAge
l_hdl_fc	Free cholesterol in large HDL	0.9293	0.0332	0.9279	0.0279	derived measures
l_ldl_p	Concentration of large LDL particles	0.9293	0.0335	0.9365	0.024	derived measures
l_vldl_ce	Cholesteryl esters in large VLDL	0.9298	0.0175	0.9317	0.0111	derived measures
estc	Total esterified cholesterol	0.9342	0.0317	0.9416	0.0203	derived measures
s_vldl_ce	Cholesteryl esters in small VLDL	0.9356	0.0257	0.9382	0.023	derived measures
idl_tg_percentage	Triglycerides to total lipids ratio in IDL	0.9366	0.0191	0.9419	0.0186	derived measures
s_hdl_tg_percentage	Triglycerides to total lipids ratio in small HDL	0.9376	0.0266	0.9382	0.0302	derived measures
xl_vldl_pl	Phospholipids in very large VLDL	0.9385	0.0308	0.9451	0.0246	derived measures
serum_c	Total cholesterol	0.9386	0.0332	0.9467	0.0192	MetaboAge
tg_pg	Ratio of triglycerides to phosphoglycerides	0.9399	0.0219	0.9452	0.0167	derived measures
val	Valine	0.9424	0.0309	0.9454	0.0261	MetaboAge and mortScore
l_hdl_ce	Cholesteryl esters in large HDL	0.9426	0.0271	0.9466	0.0191	derived measures
ace	Acetate	0.9426	0.0345	0.956	0.0362	MetaboAge
idl_p	Concentration of IDL particles	0.9427	0.0221	0.9419	0.0193	derived measures
vldl_d	Average diameter for VLDL particles	0.946	0.026	0.9493	0.0224	MetaboAge and mortScore
hdl_d	Average diameter for HDL particles	0.946	0.0207	0.9543	0.0091	MetaboAge
l_hdl_c	Cholesterol in large HDL	0.9463	0.0263	0.9533	0.0147	derived measures
s_vldl_tg	Triglycerides in small VLDL	0.9469	0.0178	0.9489	0.0173	derived measures
l_vldl_c	Cholesterol in large VLDL	0.9478	0.0135	0.9507	0.0119	derived measures
idl_pl	Phospholipids in IDL	0.9488	0.0237	0.9535	0.0101	derived measures
l_vldl_fc	Free cholesterol in large VLDL	0.9502	0.0151	0.9519	0.0177	derived measures
hdl_c	HDL cholesterol	0.9502	0.0211	0.9539	0.0141	MetaboAge
l_hdl_p	Concentration of large HDL particles	0.9508	0.0225	0.956	0.0138	derived measures
m_vldl_tg	Triglycerides in medium VLDL	0.957	0.0134	0.9587	0.0085	derived measures
s_vldl_p	Concentration of small VLDL particles	0.9586	0.0129	0.9628	0.0076	derived measures
apob	Apolipoprotein B	0.9591	0.0183	0.9638	0.0105	MetaboAge

apob_apoa1	Ratio of apolipoprotein B to apolipoprotein A1	0.9593	0.0124	0.9601	0.0104	derived measures
s_vldl_l	Total lipids in small VLDL	0.9601	0.0136	0.9648	0.0085	MetaboAge
xl_vldl_l	Total lipids in very large VLDL	0.9607	0.016	0.9617	0.0185	derived measures
l_hdl_pl	Phospholipids in large HDL	0.9609	0.0184	0.9633	0.015	derived measures
xl_vldl_p	Concentration of very large VLDL particles	0.9609	0.0149	0.9606	0.0185	derived measures
remnant_c	Remnant cholesterol (non-HDL, non-LDL - cholesterol)	0.9616	0.0176	0.9633	0.0187	derived measures
l_vldl_pl	Phospholipids in large VLDL	0.9623	0.014	0.9642	0.015	derived measures
pyr	Pyruvate	0.9625	0.0208	0.9633	0.0177	derived measures
xl_vldl_tg	Triglycerides in very large VLDL	0.963	0.0143	0.9616	0.0205	derived measures
gp	Glycoprotein acetyls	0.9643	0.0147	0.9686	0.0144	MetaboAge and mortScore
l_hdl_l	Total lipids in large HDL	0.9646	0.0164	0.9675	0.01	derived measures
ile	Isoleucine	0.9661	0.0173	0.9688	0.0159	MetaboAge and mortScore
s_vldl_c	Cholesterol in small VLDL	0.9669	0.0138	0.9699	0.0126	derived measures
l_vldl_tg	Triglycerides in large VLDL	0.968	0.0146	0.9733	0.0087	derived measures
lac	Lactate	0.9714	0.0202	0.9767	0.0119	MetaboAge and mortScore
crea	Creatinine	0.9725	0.0141	0.979	0.0145	MetaboAge
l_vldl_l	Total lipids in large VLDL	0.9725	0.0085	0.9751	0.0052	derived measures
serum_tg	Total triglycerides	0.9742	0.0093	0.9752	0.0095	MetaboAge
l_vldl_p	Concentration of large VLDL particles	0.975	0.0065	0.9767	0.0041	derived measures
tyr	Tyrosine	0.9781	0.0096	0.9798	0.0077	MetaboAge
vldl_c	VLDL cholesterol	0.9787	0.0099	0.9801	0.0081	MetaboAge
gln	Glutamine	0.9812	0.0098	0.9845	0.0067	MetaboAge
leu	Leucine	0.9844	0.0075	0.9853	0.0055	MetaboAge and mortScore
ala	Alanine	0.9851	0.0123	0.9914	0.0054	MetaboAge
vldl_tg	Triglycerides in VLDL	0.9853	0.0054	0.9863	0.0052	derived measures
glc	Glucose	0.997	0.0022	0.9972	0.0016	MetaboAge and mortScore



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## METABOLOMICS CONSORTIUM

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## Cohort Description

### Alpha Omega Cohort

The Alpha Omega Cohort consists of 4,837 Dutch men and women aged 60-80 years with a clinically diagnosed myocardial infarction <10 years before study enrollment. Baseline examinations and blood sampling took place between 2002 and 2006, after which the cohort has been followed up for cause-specific mortality. During the first 3 years of follow-up, patients participated in an intervention study with omega-3 fatty acids (Alpha Omega Trial) [1,2]. For the present analysis, a random sample of 600 patients was selected, who were followed up through Statistics Netherlands (CBS) until the 1st of January 2014. Metabolites were successfully quantified in EDTA plasma samples of 568 patients. The Alpha Omega Cohort is registered with clinicaltrials.gov (Identifier: NCT03192410).

1. Geleijnse JM, Giltay EJ, Schouten EG, et al. Effect of low doses of n-3 fatty acids on cardiovascular diseases in 4,837 post-myocardial infarction patients: design and baseline characteristics of the Alpha Omega Trial. *Am Heart J* 2010;159:539-46 e2.
2. Kromhout D, Giltay EJ, Geleijnse JM, Alpha Omega Trial G. n-3 fatty acids and cardiovascular events after myocardial infarction. *N Engl J Med* 2010;363:2015-26.

### Amsterdam Dementia Cohort

The Amsterdam Dementia Cohort is an ongoing study including patients who visit the memory clinic of the Alzheimer center of the VU University Medical Center [1]. At baseline, all subjects receive a diagnostic assessment including medical history taking, physical and neurological examination, neuropsychological investigation, standard laboratory tests of blood and cerebrospinal fluid, electroencephalogram and brain magnetic resonance imaging. Clinical diagnosis is made in consensus based, multidisciplinary meetings. For the present metabolomics analysis, 1,473 plasma samples were selected based on available volume in our biobank. In this cohort 45% is female and mean±SE age of 64±9. Date of enrollment was between 2001-2014.

1. van der Flier, W.M., et al., Optimizing patient care and research: the Amsterdam Dementia Cohort. *J Alzheimers Dis*, 2014. 41(1): p. 313-27.

### BIOMARCS

The BIOMarker study to identify the Acute risk of a Coronary Syndrome (BIOMArCS) was designed to study the relation between temporal changes in cardiovascular biomarkers and ischemic cardiovascular events in patients discharged after acute coronary syndrome (ACS) admission.[1] 844 ACS patients were enrolled in 18 hospitals in The Netherlands. Venipuncture was scheduled at 19 regular intervals during a year. 45 Patients (cases) reached the study endpoint of repeat ACS within one year. BIOMArCS was approved by the institutional review committees of the participating hospitals. All patients gave informed consent.

1. Rohit M Oemrawsingh et al. , Cohort profile of BIOMArCS: the BIOMarker study to identify the Acute risk of a Coronary Syndrome-a prospective multicentre biomarker study conducted in the Netherlands; *BMJ Open*. 2016 Dec 23;6(12):e012929. doi: 10.1136/bmjopen-2016-012929.

### LUMINA

The Leiden University Migraine Neuro-Analysis (LUMINA) cohort currently consists of over 6,700 male and female participants aged 18-88 years. LUMINA participants are recruited through a dedicated, nationwide website (<http://www.lumc.nl/org/hoofdpijn-onderzoek/onderzoek>) inviting Dutch migraine patients and non-migraine controls to participate in migraine research. Participants recruited through the website were asked to complete a screening questionnaire, previously validated to diagnose migraine [1]. The questionnaire was validated by a semi-structured telephone interview, in accordance with the International Classification of Headache Disorders (ICDH-3β)[2]. In addition, patients attending the Leiden University Medical Centre (LUMC) dedicated headache clinic were invited to participate. Migraine patients recruited through the headache clinic were diagnosed with migraine by a neurologist specialized in headache. For the present metabolomics analysis, a sample of migraine patients and non-migraine controls was selected, who were participating in several LUMINA sub cohorts (CSF, MRS, and CHARM cohorts) between 2008-2014. Migraine diagnosis was confirmed by the study-physician at the day of blood draw

resulting in the collection of 564 blood (EDTA/Serum) samples from 432 individual participants used for metabolite quantification.

1. van Oosterhout WPJ, Weller CM, Stam AH, et al. Validation of the web-based LUMINA questionnaire for recruiting large cohorts of migraineurs. *Cephalalgia*. 2011;31:1359–1367. doi: 10.1177/0333102411418846.

2. Headache Classification Committee of the International Headache Society (IHS). The International Classification of Headache Disorders, 3rd edition (beta version). *Cephalalgia*. 2013;33:629–808. doi: 10.1177/0333102413485658.

## **CHECK**

CHECK (Cohort Hip & Cohort Knee) is a prospective, 10-year follow-up, observational cohort study of 1002 people aged between 45 and 65 years at the time of inclusion, with pain in their knee(s) and/or hip(s), who had never or not longer than 6 months ago consulted a physician for these complaints [1]. Blood samples were taken non-fasted. Hip and knee radiographs were obtained multiple times throughout follow-up and scored pairwise according to the Kellgren & Lawrence (KL) scoring system. When scored pairwise, these people did not have obvious radiographic knee or hip OA at baseline (i.e. KLO or 1).

1. Wesseling J, Dekker J, van den Berg WB, Bierma-Zeinstra SM, Boers M, Cats HA, et al. CHECK (Cohort Hip and Cohort Knee): similarities and differences with the Osteoarthritis Initiative. *Ann Rheum Dis* 2009;68:1413-9.

## **CODAM**

The Cohort on Diabetes and Atherosclerosis Maastricht [1] (CODAM) is a prospective, observational study that consists of 574 individuals who were selected from a larger population-based cohort [2]. Inclusion of participants into CODAM was based on a moderately increased risk to develop cardiometabolic diseases, such as type 2 diabetes and/or cardiovascular disease. Participants were included if they were of Caucasian descent and over 40 yrs of age and additionally met at least one of the following criteria: increased BMI ( $>25 \text{ kg/m}^2$ ), a positive family history of type 2 diabetes, a history of gestational diabetes and/or glycosuria, or use of anti-hypertensive medication. The CODAM baseline measurements were done between 2000 and 2002 ( $n=352$  men, 222 women, age  $59.6 \pm 7.0$  yrs). After a median of 7.0 years (interquartile range 6.9–7.1), 495 participants were included in the follow-up measurements [3]. Metabolites were obtained fasting EDTA plasma samples of all participants at baseline and at follow-up in those participants who had type 2 diabetes at baseline ( $n=110$ ).

1. van Greevenbroek, M. M. J. et al. The cross-sectional association between insulin resistance and circulating complement C3 is partly explained by plasma alanine aminotransferase, independent of central obesity and general inflammation (the CODAM study). *Eur. J. Clin. Invest.* 41, 372–379 (2011). doi: 10.1111/j.1365-2362.2010.02418.x

2. Van Dam, R. M., Boer, J. M., Feskens, E. J. M. & Seidell, J. C. Parental history of diabetes modifies the association between abdominal adiposity and hyperglycemia. *Diabetes Care* 24, 1454–1459 (2001).

3. Wlazlo N, van Greevenbroek MM, Ferreira I, Feskens EJ, van der Kallen CJ, Schalkwijk CG, Bravenboer B, Stehouwer CD. Complement factor 3 is associated with insulin resistance and with incident type 2 diabetes over a 7-year follow-up period: the CODAM Study. *Diabetes Care* 37, 1900-1909 (2014). doi: 10.2337/dc13-2804

## **The Maastricht Study (TMS)**

The Maastricht Study [1] is an observational prospective population-based cohort study enriched with T2DM individuals. Eligible for participation were individuals aged between 40 and 75 years and living in the southern part of the Netherlands (municipalities Maastricht, Margraten-Eijsden, Meerssen and Valkenburg; Maastricht and Heuvelland in the province of Limburg).

1. Schram MT1 et al. The Maastricht Study: an extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities. *Eur J Epidemiol*. 2014 Jun;29(6):439-51. doi: 10.1007/s10654-014-9889-0. Epub 2014 Apr 23.

## **The Hoorn Diabetes Care System cohort study (DCS)**

The DCS cohort currently consists of approximately 13.000 people with type 2 diabetes. The DCS provides routine diabetes care to people with type 2 diabetes living in the West-Friesland region of the Netherlands [1]. People treated by the DCS visit the DCS research center annually, during which blood is drawn in the fasting state for routine biochemistry. Furthermore, all participants get a full medical exam, advice about their health and treatment and receive education on their disease during their annual visits to the DCS research center. In addition, patients are invited to join our research and biobanking studies (n=5,000+). From the DCS biobank we included for this study a random cross-sectional sample for which a baseline plasma sample and yearly follow-up data were available (n=750). For case-control analyses this sample was supplemented with individuals selected for the inability to reach the glycaemic target (HbA1c > 53 mmol/mol) and/or suffering from diabetic complications (n=245). Samples were collected in 2008/2009 and stored at -80 degrees Celsius until analysis. Metabolites were successfully quantified in fasting EDTA plasma samples from 995 individuals.

[1] van der Heijden AA, Rauh SP, Dekker JM, Beulens JW, Elders P, 't Hart LM, Rutters F, van Leeuwen N, Nijpels G: The Hoorn Diabetes Care System (DCS) cohort. A prospective cohort of persons with type 2 diabetes treated in primary care in the Netherlands. *BMJ Open* 2017;7:e015599

Website: [www.hoornstudies.com](http://www.hoornstudies.com)

### **Erasmus Rucphen Family study (ERF)**

The Erasmus Rucphen Family is a family-based study that includes inhabitants of a genetically isolated community in the South-West of the Netherlands [1]. The goal of the study is to identify the risk factors in the development of complex disorders. Study population includes approximately 3,000 individuals who are living descendants of a limited number of founders living in the 19th century [1]. Metabolomics measurements were quantified from fasted EDTA plasma samples using Nightingale Health platform. All data were collected between 2002 and 2005. Metabolomics measurements were available for 1,402 participants from the ERF.

1. Pardo, Luba M., et al. "The effect of genetic drift in a young genetically isolated population." *Annals of human genetics* 69.3 (2005): 288-295.

### **Rotterdam Study (RS)**

The Rotterdam Study is a prospective, population-based cohort study among individuals living in the well-defined Ommoord district in the city of Rotterdam in The Netherlands [1]. The aim of the study is to determine the occurrence of cardiovascular, neurological, ophthalmic, endocrine, hepatic, respiratory, locomotor, dermatological, otolaryngological, and psychiatric diseases in elderly people. The cohort was initially defined in 1990 among approximately 7,983 persons, aged 55 years and older, who underwent a home interview and extensive physical examination at the baseline and during follow-up visits every 3-4 years (RS-I) [1]. Cohort was extended in 2000/2001 (RS-II, 3,011 individuals aged 55 years and older) and 2006/2008 (RS-III, 3,932 subjects, aged 45 and older). As of 2008, Rotterdam Study comprised 14,926 subjects. Written informed consent was obtained from all participants and the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, approved the study. Metabolomics measurements were quantified in fasted EDTA plasma samples using Nightingale Health platform. Metabolomics measurements were available for 2,986 participants from RS-I, 591 participants from RS-II (n=591) and 1,787 participants from RS-III.

1. Ikram, M. Arfan, et al. "The Rotterdam Study: 2018 update on objectives, design and main results." *European Journal of Epidemiology* 32.9 (2017): 807-850.

### **FUNCTGENOMICS**

The 500 Functional Genomics (500FG [1]) project consists of 534 adult healthy volunteers sampled between July 2013 and December 2014. Inclusion criteria were >18 years of age and Western European descent. Exclusion criteria were pregnancy/breastfeeding, chronic or acute disease at the time of assessment, and use of chronic or acute medication during the last month before the study. After visiting the hospital to donate blood, volunteers received an extensive online questionnaire about lifestyle, diet, and disease history. Upon analyzing the questionnaire data we excluded 45 volunteers as they were under medication, non-European descent, or had kidney disease or diabetes mellitus.

1. Schirmer et al. Linking the Human Gut Microbiome to Inflammatory Cytokine Production Capacity. *Cell*. 2016 Nov 3;167(4):1125-1136.e8. doi: 10.1016/j.cell.2016.10.020.

### **GARP**

The GARP cohort (N=217) consists of patients with advanced radiographic OA at two or more joint sites of hand, spine, knee or hip. Follow-up was performed at 5 years, at which radiographs for hip, knee and hand were scored pairwise using the OARSI Atlas and the KL scoring system. Matched to the GARP study, a normal reference control group (NORREF) was collected using the same protocol and included in this study as controls [1-3]. Blood was collected non-fasted.

1. Riyazi N, Meulenbelt I, Kroon HM, Roodenrys KH, Hellio le Graverand MP, Rosendaal FR, et al. Evidence for familial aggregation of hand, hip, and spine but not knee osteoarthritis in siblings with multiple joint involvement: the GARP study. *Ann Rheum Dis* 2005;64:438-43.

2. Meulenbelt I, Kloppenburg M, Kroon HM, Houwing-Duistermaat JJ, Garnero P, Hellio-Le Graverand MP, et al. Clusters of biochemical markers are associated with radiographic subtypes of osteoarthritis (OA) in subject with familial OA at multiple sites. *The GARP study. Osteoarthritis Cartilage* 2007;15:379-85.

3. Bijsterbosch J, Meulenbelt I, Watt I, Rosendaal FR, Huizinga TW, Kloppenburg M. Clustering of hand osteoarthritis progression and its relationship to progression of osteoarthritis at the knee. *Ann Rheum Dis* 2014;73:567-72.

### **HELIUS study**

The HELIUS study is a prospective cohort study among the largest ethnic groups living in Amsterdam, the Netherlands. The aim of the HELIUS study is to investigate the causes of (the unequal burden of) diseases across ethnic groups, focusing on three disease categories: cardiovascular diseases, mental health and infectious diseases [1]. Between 2011-2015, a total 24,789 participants (men and women aged 18-70 years) were included at baseline. Similar-sized samples of individuals of Dutch, African Surinamese, South-Asian Surinamese, Ghanaian, Turkish and Moroccan origin were included. Participants filled in an extensive questionnaire and underwent a physical examination that included the collection of biological samples (biobank). Follow-up data is obtained by linkages with existing registries (e.g. hospital data, insurance data) and will be obtained by repeated measurements [2]. Metabolites were quantified in EDTA plasma samples of 500 African origin participants with (pre)diabetes (235 African Surinamese, 265 Ghanaian participants).

[1] K Stronks, MB Snijder, RJ Peters, M Prins, AH Schene, AH Zwinderman. Unravelling the impact of ethnicity on health in Europe: the HELIUS study. *BMC Public Health* 2013;13:402.

[2] MB Snijder, H Galenkamp, M Prins, EM Derks, RJ Peters, AH Zwinderman, K Stronks. Cohort Profile: the Healthy Life in an Urban Setting (HELIUS) study. *BMJ Open* (in press).

**Website:** [www.heliusstudie.nl](http://www.heliusstudie.nl)

### **LIFELINES-DEEP**

The LifeLines-DEEP cohort is a subset of the Dutch general population cohort LifeLines. Both LifeLines and LifeLines DEEP have been previously described [1-3]. In summary, LifeLines is a three-generation observational follow-up study, which was set up to investigate universal risk factors and their modifiers for multifactorial diseases. Since 2006, approximately 167,000 individuals from the general population residing in the three northern provinces of the Netherlands participate in the study. All participants will be followed-up prospectively for at least 30 years. Participants regularly undergo physical examinations and fill in extensive questionnaires. In addition, blood and urine samples are collected. Each participant is asked to fill in health, lifestyle, and quality-of-life questionnaires every 1.5 years, whereas each participant is invited for a follow-up visit to a Lifelines clinic every 5 years [1,2]. LifeLines-DEEP comprises 1,539 participants (636 males and 903 females, age range 18–84 years). This study was set up for the more detailed phenotyping and omics profiling. For analysis of the genome, epigenome, transcriptome, microbiome, metabolome and other biological levels, additional biomaterials were collected, including additional blood, exhaled air and fecal samples, as well as responses to gastrointestinal health [3].

The current metabolomics study included the baseline information and plasma samples of LifeLines-DEEP participants. EDTA plasma samples were collected after overnight fasting. Peripheral blood samples were drawn by venipuncture from the median cubital vein and subsequently placed at 4°C. Transport of the samples from the research site to the LifeLines laboratory in Groningen was under tightly controlled and continuously monitored conditions. At the LifeLines site, plasma was prepared and aliquoted and stored at -80°C. The samples underwent two freeze-thaw cycles prior to shipment to Brainshake for metabolome analysis [1]. With some sample drop-off, this study eventually included 1,440 LifeLines-DEEP participants. The LifeLines DEEP study was approved by the institutional ethics review board of University Medical Center Groningen (ref. M12.113965).

1. Scholtens, S. et al. Cohort Profile: LifeLines, a three-generation cohort study and biobank. *Int. J. Epidemiol.* 44, 1172–1180 (2015).

2. Stolk, R. P. et al. Universal risk factors for multifactorial diseases: LifeLines: A three-generation population-based study. *Eur. J. Epidemiol.* 23, 67–74 (2008).

3. Tigchelaar E. F. et al., Cohort profile: LifeLines DEEP, a prospective, general population cohort study in the northern Netherlands: study design and baseline characteristics. *BMJ Open* 5, e006772 (2015).

**Website:** <https://www.lifelines.nl/>

### **Leiden Longevity Study (LLS)**

The Leiden Longevity Study (LLS) consists of 421 long-lived families of European descent. Families were included if at least two long-lived siblings were alive and fulfilled the age criterion of 89 years or older for males and 91 years or older for females, representing <0.5% of the Dutch population in 2001 [1]. In total, 944 long-lived proband siblings (mean age = 94 years, range = 89-104), 1671 offspring (mean age = 61 years, range = 39-81) and 744 spouses thereof (mean age = 60 years, range = 36-79) were included. Registry-based follow-up until the 27<sup>th</sup> of October 2016 was available for all participants. Metabolites were successfully quantified in 843 nonagenarians [LLS\_SIBS], 1157 of their offspring and 684 controls (LLS\_PAROFF) using non-fasted EDTA plasma samples.

1. M. Schoenmaker et al. Evidence of genetic enrichment for exceptional survival using a family approach: the Leiden Longevity Study. *Eur J Hum Genet.* 2006 Jan;14(1):79-84.

### **NESDA**

The Netherlands Study of Depression and Anxiety (NESDA) is an ongoing observational longitudinal cohort study on the long term course and consequences of depressive and anxiety disorders [1]. Between September 2004 and February 2007, 2,981 participants (1,979 females, 1,002 males) aged 18 through 65 years were included. They were recruited through different settings (community, primary care and specialized mental health clinics) in order to obtain a representative sample of persons with depressive and/or anxiety disorders (in lifetime, n=2,329) and without depressive and/or anxiety disorders (n=652). At baseline, participants completed the 4-hour baseline assessment, which included a face-to-face interview, written questionnaires, and biological measurements. Follow-up visits to the research center have now been finished 2, 4, 6 and 9 years after baseline, with response rates of n=2,596, n=2,402, n=2,256 and n=2,069, respectively. The research protocol was approved by the Ethical Committee of the participating centers, and all participants provided written informed consent. During the baseline interview, EDTA plasma samples were collected and stored in aliquots at -85°C until further analysis. Participants were instructed to have an overnight fast before blood collection. Metabolites in these blood samples were analyzed in 2 batches (April and December 2014, respectively) by Brainshake Ltd./Nightingale Health, Helsinki, Finland.

[1] Penninx BWJH, Beekman ATF, Smit JH, Zitman FG, Nolen WA, Spinhoven P, et al. The Netherlands Study of Depression and Anxiety (NESDA): rationale, objectives and methods. *Int J Methods Psychiatr Res* 2008;17:121–40. doi: 10.1002/mpr.256.

**Website:** <http://www.nesda.nl>

### **PROspective Study of Pravastatin in the Elderly at Risk (PROSPER)**

The PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) trial design has been published [1,2]. In brief, 5,804 elderly adults (70-82 years old) were enrolled. This was a double-blind, randomised placebo controlled trial investigating the benefit of pravastatin (40 mg/day) in elderly individuals at risk of CVD. Participants were identified

in the primary care setting from 3 centres: Glasgow, Scotland; Cork, Ireland or Leiden, the Netherlands. All participants had high-normal to high cholesterol (4.0-9.0 mmol/L) at baseline. Additionally 50% of patients had evidence of vascular disease (physician diagnosed stable angina, stroke, transient ischaemic attack (TIA) or myocardial infarction (MI)) and the remaining 50% of patients had high risk of vascular disease as they had either hypertension, diabetes or were smokers. The primary outcome measure of PROSPER was a composite CVD outcome. In the current study the endpoint of interest was all-cause mortality. Patients were recruited between December 1997 and May 1999 and the mean follow-up period was 3.2 years. Fasting venous blood samples were collected at baseline and at 3-month intervals and biobanked at -80°C. For the present study previously unfrozen 6-month post-randomisation samples were used, employing the study as a cohort study and adjusting for randomised treatment in the analyses. Metabolites were successfully quantified in 5,329 individuals.

1. Shepherd J, Blauw GJ, Murphy MB, Cobbe SM, Bollen EL, Buckley BM, et al. The design of a prospective study of Pravastatin in the Elderly at Risk (PROSPER). PROSPER Study Group. PROSpective Study of Pravastatin in the Elderly at Risk. *Am J Cardiol* 1999 Nov 15;84(10):1192-7. PMID: 10569329

2. Shepherd J, Blauw GJ, Murphy MB, Bollen EL, Buckley BM, Cobbe SM, et al. Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. *Lancet* 2002 Nov 23;360(9346):1623-30. PMID:12457784

#### **LUMC Arthroplasty studies:**

The LUMC arthroplasty studies (N=462) consist of participants of the RAAK, TacTics (NTR309) and TOMaat (NTR303) studies [1, 2]. These cross-sectional studies included OA patients who received THA or TKA. Since all participants underwent THA/TKA, all patients are considered as end-stage OA and included in the cross-sectional OA prevalence analysis. Blood samples were collected during surgery while patients were fasted.

[1] Ramos YF, den Hollander W, Bovee JV, Bomer N, van der Breggen R, Lakenberg N, et al. Genes involved in the osteoarthritis process identified through genome wide expression analysis in articular cartilage; the RAAK study. *PLoS One* 2014;9:e103056.

[2] So-Osman C, Nelissen RG, Koopman-van Gemert AW, Kluyver E, Poll RG, Onstenk R, et al. Patient blood management in elective total hip- and knee-replacement surgery (Part 1): a randomized controlled trial on erythropoietin and blood salvage as transfusion alternatives using a restrictive transfusion policy in erythropoietin-eligible patients. *Anesthesiology* 2014;120:839-51.

#### **STEMI-GIPS-III**

The Glycometabolic Intervention as Adjunct to Primary Coronary Intervention in ST Elevation Myocardial Infarction (GIPS-III) study is a placebo-controlled randomized clinical trial to evaluate the effect of metformin therapy on left ventricular function in 380 non-diabetic ST-elevated myocardial infarction (STEMI) patients aged 23-90. Patients were included between 2011 and 2013. Blood samples were collected at several time points after inclusion. During the 4-month treatment period, patients received metformin or placebo. 4 months after randomization, left ventricular ejection fraction (LVEF) was measured by cardiac MRI. In addition, patients were followed up for major adverse cardiac events (death, recurrent MI, target lesion revascularization), stroke, non-elective hospitalizations for chest pain or heart failure, all recurrent coronary interventions and internal cardiac defibrillator implantations. Metabolic profiling was assessed in EDTA plasma samples collected at baseline (hospital admission), 24 h post-MI and 4 months post-MI. GIPS-III is registered with clinicaltrials.gov (ID: NCT01217307)

1. Eppinga RN, Kofink D, Dullaart RP, Dalmeijer GW, Lipsic E, van Veldhuisen DJ, van der Horst IC, Asselbergs FW, van der Harst P. Effect of Metformin on Metabolites and Relation With Myocardial Infarct Size and Left Ventricular Ejection Fraction After Myocardial Infarction. *Circ Cardiovasc Genet*. 2017 Feb;10(1).

2. Eppinga RN, Hartman MH, van Veldhuisen DJ, Lexis CP, Connelly MA, Lipsic E, van der Horst IC, van der Harst P, Dullaart RP. Effect of Metformin Treatment on Lipoprotein Subfractions in Non-Diabetic Patients with Acute Myocardial Infarction: A Glycometabolic Intervention as Adjunct to Primary Coronary Intervention in ST Elevation Myocardial Infarction (GIPS-III) Trial. *PLoS One*. 2016 Jan 25;11(1):e0145719.

3. Lexis CP, van der Horst-Schrivers AN, Lipsic E, Valente MA, Muller Kobold AC, de Boer RA, van Veldhuisen DJ, van der Harst P, van der Horst IC. The effect of metformin on cardiovascular risk profile in patients without diabetes

presenting with acute myocardial infarction: data from the Glycometabolic Intervention as adjunct to Primary Coronary Intervention in ST Elevation Myocardial Infarction (GIPS-III) trial. *BMJ Open Diabetes Res Care*. 2015 Dec 11;3(1):e000090.

4. Lexis CP, van der Horst IC, Lipsic E, Wieringa WG, de Boer RA, van den Heuvel AF, van der Werf HW, Schurer RA, Pundziute G, Tan ES, Nieuwland W, Willemsen HM, Dorhout B, Molmans BH, van der Horst-Schrivers AN, Wolffenbuttel BH, ter Horst GJ, van Rossum AC, Tijssen JG, Hillege HL, de Smet BJ, van der Harst P, van Veldhuisen DJ; GIPS-III Investigators. Effect of metformin on left ventricular function after acute myocardial infarction in patients without diabetes: the GIPS-III randomized clinical trial. *JAMA*. 2014 Apr 16;311(15):1526-35.

5. Lexis CP, van der Horst IC, Lipsic E, van der Harst P, van der Horst-Schrivers AN, Wolffenbuttel BH, de Boer RA, van Rossum AC, van Veldhuisen DJ, de Smet BJ; GIPS-III Investigators. Metformin in non-diabetic patients presenting with ST elevation myocardial infarction: rationale and design of the glycometabolic intervention as adjunct to primary percutaneous intervention in ST elevation myocardial infarction (GIPS)-III trial. *Cardiovasc Drugs Ther*.

### UCORBIO

The Utrecht Coronary Biobank Study (UCORBIO) enrolled 2,591 patients aged 18-93 who underwent coronary angiography for any indication at the UMC Utrecht. Baseline assessment and blood sampling took place between 2011 and 2014. Patients were followed up for the occurrence of major adverse cardiovascular events (stroke, myocardial infarction, coronary revascularization, death). During the follow-up period (maximum: 3 years), patients completed questionnaires every year to obtain information on hospital admissions. General practitioners and hospitals were contacted to confirm reported cardiovascular events. EDTA samples of 1,198 patients were selected for metabolic profiling. UCORBIO is registered with [clinicaltrials.gov](https://clinicaltrials.gov) (ID: NCT02304744).

1. Gijsberts CM, Santema BT, Asselbergs FW, de Kleijn DP, Voskuil M, Agostoni P, Cramer MJ, Vaartjes I, Hoefer IE, Pasterkamp G, den Ruijter HM. Women Undergoing Coronary Angiography for Myocardial Infarction or Who Present With Multivessel Disease Have a Poorer Prognosis Than Men. *Angiology*. 2016 Jul;67(6):571-81.

2. Gijsberts CM, Agostoni P, Hoefer IE, Asselbergs FW, Pasterkamp G, Nathoe H, Appelman YE, de Kleijn DP, den Ruijter HM. Gender differences in health-related quality of life in patients undergoing coronary angiography. *Open Heart*. 2015 Aug 27;2(1):e000231.

3. Gijsberts CM, Gohar A, Ellenbroek GH, Hoefer IE, de Kleijn DP, Asselbergs FW, Nathoe HM, Agostoni P, Rittersma SZ, Pasterkamp G, Appelman Y, den Ruijter HM. Severity of stable coronary artery disease and its biomarkers differ between men and women undergoing angiography. *Atherosclerosis*. 2015 Jul;241(1):234-40.

### VUNTR

Since 1987, the Netherlands Twin Register is collecting (longitudinal) data in young and adult twins and their families [1,2]. The rich phenotypic longitudinal information that has been collected extends from lifestyle, exposures, personality and demographics information to mental and somatic health. In subgroups information on autonomic and central nervous system function, biomarkers and gene expression, epigenetics and genotyping is available. A 2015 estimate is that, since initiating the NTR, ~25% of all twins and multiples in the Netherlands participated in NTR research projects. Longitudinal information for over 200,000 participants (twins, multiples and family members) was collected over multiple NTR research projects. Data collection is ongoing. A pdf of nearly all published papers may be found at the NTR website.

As part of a Netherlands Twin Register (NTR) biobank project (BB1), 9,530 participants from 3,477 families were visited at home between January 2004 and July 2008 for collection of blood samples [3]. A second project (BB2) collected blood samples in 517 subjects from January 2011 to December 2011, including 210 MZ twin pairs and 64 twin-spouse pairs [4]. Visits were scheduled between 7:00 and 10:00 am and fertile women were bled on day 2-4 of the menstrual cycle, or in their pill-free week. Body composition was measured and information about physical health and lifestyle (e.g. smoking and drinking behavior, exercise, medication use) was obtained. For more detailed information about the methodology of the NTR Biobank study, see [3]. The NTR studies were approved by the Central Ethics Committee on Research involving human subjects of the VUMC, Amsterdam, an Institutional Review Board certified by the US Office of Human Research Protections (IRB number IRB-2991 under Federal wide Assurance-3703; IRB/institute codes, NTR 03-180). All subjects provided written informed consent. Subject were selected who were part of NTR biobank and who in general had rich phenotyping data available.



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2. Willemsen G, Vink JM, Abdellaoui A, den Braber A, van Beek JH, Draisma HH, van Dongen J, van 't Ent D, Geels LM, van Lien R, Ligthart L, Kattenberg M, Mbarek H, de Moor MH, Neijts M, Pool R, Stroo N, Kluft C, Suchiman HE, Slagboom PE, de Geus EJ, Boomsma DI. The Adult Netherlands Twin Register: twenty-five years of survey and biological data collection. *Twin Res Hum Genet.* **2013** Feb; 16(1): 271-281. doi: [10.1017/thg.2012.140](https://doi.org/10.1017/thg.2012.140). PMID: 23298648. PMCID: PMC3739974.
3. Willemsen G, de Geus EJ, Bartels M, van Beijsterveldt CE, Brooks AI, Estourgie-van Burk GF, Fugman DA, Hoekstra C, Hottenga JJ, Kluft K, Meijer P, Montgomery GW, Rizzu P, Sondervan D, Smit AB, Spijker S, Suchiman HE, Tischfield JA, Lehner T, Slagboom PE, Boomsma DI. The Netherlands Twin Register biobank: a resource for genetic epidemiological studies. *Twin Res Hum Genet.* **2010** Jun; 13(3): 231-245. doi: [10.1375/twin.13.3.231](https://doi.org/10.1375/twin.13.3.231). PMID: 20477721.
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website: [www.tweelingenregister.org/](http://www.tweelingenregister.org/)

## HOF

The HOF study is based on the follow-up of selected births in 1943-1947 in the Amsterdam and Rotterdam Midwife training schools and the Leiden University Department of Obstetrics. The aim of the study was to assess the long-term health impact of pre-natal exposure to the Dutch famine of 1944–1945. The study includes pre-famine and post-famine births in these clinics as time controls and same-sex siblings as family controls. Selected births were traced through population registries in the Netherlands and underwent a telephone interview and extensive physical examinations in 2003-2005. They also provided blood samples and specific informed consents for further studies on risk factors for cardiovascular and metabolic disease. Metabolomic measurements were available for 971 men and women of the study.

1. Lumey LH, Stein AD, Kahn HS, van der Pal-de Bruin KM, Blauw GJ, Zybert PA, et al. Cohort profile: the Dutch Hunger Winter families study. *International Journal of Epidemiology* 2007; 36(6):1196-204.

## STABILITEIT

The STABILITEIT cohort contains subsample of the Amsterdam Dementia Cohort study (ADC). The aim of the study was to study the effects of long-term storage on the metabolic biomarkers, over a period of 14 years.

## Acknowledgements

### **Alpha Omega Cohort**

Financial support for the Alpha Omega Cohort was obtained from the Dutch Heart Foundation (grant 200T401) and the National Institutes of Health (grant R01HL076200), USA. DNA isolation was funded by BBMRI-NL (grant CP2011-18).

### **Amsterdam Dementia Cohort**

Research within the VUmc Alzheimer center is part of the neurodegeneration research program of Amsterdam Neuroscience supported by Alzheimer Nederland and Stichting VUmc fonds.

### **BIOMARCS**

BIOMArCS was funded by the Netherlands Heart Foundation (grant 2007B012), the Netherlands Heart Institute, the Working Group Cardiovascular Research Netherlands, and Eli Lilly through unrestricted research grants. The funders had no role in the design and conduct of the study; collection, management, analysis and interpretation of the data; preparation, review or approval of the manuscript; or decision to submit the manuscript for publication.

### **LUMINA**

LUMINA (i.e. its subcohorts CHARM, CSF, MRS) is supported by grants obtained from the Netherlands Organization for the Health Research and Development (ZonMw; no. 90700217) and VIDI (ZonMw; no. 91711319); the Netherlands Organisation for Scientific Research (NWO) VICI (no.918.56.602) and Spinoza prize (2009) grants; the Centre for Medical Systems Biology (CMSB) and Netherlands Consortium for Systems Biology (NCSB), both within the framework of the Netherlands Genomics Initiative (NGI)/Netherlands Organization for Scientific Research (NWO) the 7th Framework EU project EUROHEADPAIN (no. 602633).

### **CHECK**

CHECK was funded by the Dutch Arthritis Association on the lead of a steering committee comprising 16 members with expertise in different fields of OA, chaired by Professor JWJ Bijlsma and coordinated by J Wesseling. Involved are: Erasmus Medical Center Rotterdam; Kennemer Gasthuis Haarlem; Leiden University Medical Center; Maastricht University Medical Center; Martini Hospital Groningen/Allied Health Care Center for Rheumatology and Rehabilitation Groningen; Medical Spectrum Twente Enschede/Ziekenhuisgroep Twente Almelo; Reade, formerly Jan van Breemen Institute/VU Medical Center Amsterdam; St. Maartenskliniek Nijmegen; University Medical Center Utrecht, and Wilhelmina Hospital Assen.

### **CODAM**

The initiation of the CODAM study was supported by grants of the Netherlands Organisation for Scientific Research (940-35-034) and the Dutch Diabetes Research Foundation (98-901).

### **The Maastricht Study (TMS)**

This study was supported by the European Regional Development Fund via OP-Zuid, the Province of Limburg, the Dutch Ministry of Economic Affairs (grant 31O.041), Stichting De Weijerhorst (Maastricht, the Netherlands), the Pearl String Initiative Diabetes (Amsterdam, the Netherlands), CARIM School for Cardiovascular Diseases (Maastricht, the Netherlands), Stichting Annadal (Maastricht, the Netherlands), Health Foundation Limburg (Maastricht, the Netherlands) and by unrestricted grants from Janssen-Cilag B.V. (Tilburg, the Netherlands), Novo Nordisk Farma B.V. (Alphen aan den Rijn, the Netherlands) and Sanofi-Aventis Netherlands B.V. (Gouda, the Netherlands).

### **The Hoorn Diabetes Care System cohort study (DCS)**

This study has been made possible by collaboration with the Diabetes Care System West-Friesland. The authors thank participants of this study and research staff of the Diabetes Care System West-Friesland.

### **Erasmus Rucphen Family (ERF) study**

The Erasmus Rucphen Family (ERF) study has received funding from the Centre for Medical Systems Biology (CMSB) and Netherlands Consortium for Systems Biology (NCSB), both within the framework of the Netherlands Genomics Initiative (NGI)/Netherlands Organization for Scientific Research (NWO). ERF study is also a part of EUROSPAN (European Special Populations Research Network) (FP6 STREP grant number 18947 (LSHG-CT-2006-018947)); European Network of Genomic and Genetic Epidemiology (ENGAGE) from the European Community's Seventh Framework Programme (FP7/2007-2013)/grant agreement HEALTH-F4-2007-201413; "Quality of Life and Management of the Living Resources" of 5th Framework Programme (no. QLG2-CT-2002-01254); FP7 project EUROHEADPAIN (nr 602633), the Internationale Stichting Alzheimer Onderzoek (ISAO); the Hersenstichting Nederland (HSN); and the JNPD under the project PERADES (grant number 733051021, Defining Genetic, Polygenic and Environmental Risk for Alzheimer's Disease using multiple powerful cohorts, focused Epigenetics and Stem cell metabolomics). Metabolomics measurements of ERF has been funded by Biobanking and Biomolecular Resources Research Infrastructure (BBMRI)-NL (184.021.007). The ERF-follow up study is funded by CardioVasculair Onderzoek Nederland (CVON 2012-03). We are grateful to all study participants and their relatives, general practitioners and neurologists for their contributions and to P. Veraart for her help in genealogy, J. Vergeer for the supervision of the laboratory work, both S.J. van der Lee and A. van der Spek for collection of the follow-up data and P. Snijders for his help in data collection of both baseline and follow-up data.

#### **Rotterdam Study**

The Rotterdam Study is supported by the Erasmus MC University Medical Center and Erasmus University Rotterdam; The Netherlands Organisation for Scientific Research (NWO); The Netherlands Organisation for Health Research and Development (ZonMw); the Research Institute for Diseases in the Elderly (RIDE); The Netherlands Genomics Initiative (NGI); the Ministry of Education, Culture and Science; the Ministry of Health, Welfare and Sports; the European Commission (DG XII); and the Municipality of Rotterdam. The authors are grateful to the study participants, the staff from the Rotterdam Study and the participating general practitioners and pharmacists. Metabolomics measurements were funded by Biobanking and Biomolecular Resources Research Infrastructure (BBMRI)-NL (184.021.007) and the JNPD under the project PERADES (grant number 733051021, Defining Genetic, Polygenic and Environmental Risk for Alzheimer's Disease using multiple powerful cohorts, focused Epigenetics and Stem cell metabolomics).

#### **FUNCTGENOMICS**

##### **GARP**

The Leiden University Medical Centre have and are supporting the RAAK and GARP study. This study was supported by the Dutch Arthritis Foundation and Pfizer Groton, Connecticut, USA. We are indebted to drs. N. Riyazi, J. Bijsterbosch, H.M. Kroon and I. Watt for collection of data.

##### **HELIUS**

The HELIUS study is conducted by the Academic Medical Center Amsterdam and the Public Health Service of Amsterdam. Both organisations provided core support for HELIUS. The HELIUS study is also funded by the Dutch Heart Foundation, the Netherlands Organization for Health Research and Development (ZonMw), the European Union (FP-7), and the European Fund for the Integration of non-EU immigrants (EIF).

##### **LIFELINES-DEEP**

We thank the participants and staff of the Lifelines cohort for their collaboration, particularly B. Bolmer and S. Gerritsma for coordinating the Lifelines data. We thank J. Dekens and J. Arends for management and technical support and the Genomics Coordination Center for providing data infrastructure and access to high performance computing clusters. This project was funded by the Netherlands Heart Foundation (IN-CONTROL CVON grant 2012-03 and 2018-27), the grant from the Top Institute Food and Nutrition (TiFN GH001), the Netherlands Organization for Scientific Research (NWO) 024.004.017, 864.13.013, 016.178.056, 917.14.374, SPI 92-266; the European Research Council Consolidate grant 101001678; and the RuG Investment Agenda Grant Personalized Health grant.

#### **Leiden Longevity Study (LLS)**

The LLS has received funding from the European Union's Seventh Framework Programme (FP7/2007-2011) under grant agreement n° 259679. This study was supported by a grant from the Innovation-Oriented Research Program on Genomics (SenterNovem IGE05007), the Centre for Medical Systems Biology, and the Netherlands Consortium for Healthy Ageing (grants 05040202 and 050-060-810), all in the framework of the Netherlands Genomics Initiative, Netherlands Organization for Scientific Research (NWO), Unilever Colworth, and by BBMRI-NL, a Research Infrastructure financed by the Dutch government (NWO 184.021.007).

#### **NESDA**

The infrastructure for the NESDA study ([www.nesda.nl](http://www.nesda.nl)) is funded through the Geestkracht program of the Netherlands Organisation for Health Research and Development (ZonMw, grant number 10-000-1002) and financial contributions by participating universities and mental health care organizations (VU University Medical Center, GGZ inGeest, Leiden University Medical Center, Leiden University, GGZ Rivierduinen, University Medical Center Groningen, University of Groningen, Lentis, GGZ Friesland, GGZ Drenthe, Rob Giel Onderzoekscentrum).

#### **PROspective Study of Pravastatin in the Elderly at Risk (PROSPER)**

The PROSPER study was supported by an investigator initiated grant obtained from Bristol-Myers Squibb. Prof. Dr. J. W. Jukema is an Established Clinical Investigator of the Netherlands Heart Foundation (grant 2001 D 032). PROSPER was supported by the European Federation of Pharmaceutical Industries Associations (EFPIA), Innovative Medicines Initiative Joint undertaking, European Medical Information Framework (EMIF) grant number 115372 and the European Commission under the Health Cooperation Work Programme of the 7th Framework Programme (Grant number 305507) "Heart 'omics' in AGEing" (HOMAGE).

#### **The LUMC arthroplasty studies**

This was a combination of TACTICS, TOMAAT and RAAK cohorts. TACTICS was funded by The Dutch Board of Health Care Insurances (College voor Zorgverzekeringen; OG99/023) and Sanquin Blood Bank. Involved were Prof. dr R.G.H.H. Nelissen, MD, Prof. dr A. Brand, MD, Leiden University Medical Centre; R.L. te Slaa MD, Reinier de Graaf Gasthuis, Delft; Dr R.G. Poll MD, Slotervaart ziekenhuis, Amsterdam; Dr K.M. Veenstra Franciscus ziekenhuis, Rotterdam and Prof. dr D. van Rhenen Sanquin Blood Bank, Rotterdam. Funding for the TOMAAT-study was received from ZonMW (06-601) and Sanquin Blood Supply (03-002), the Netherlands. Clinical Trial Number: ISRCTN96327523 ([controlled-trials.com](http://controlled-trials.com)) and NTR 303 (Dutch Trial Register). The RAAK study was supported by the Leiden University Medical Centre. Furthermore, the molecular studies performed within to the RAAK study has received funding from the Dutch Arthritis Association (DAA\_10\_1-402), Biobanking and BioMolecular resources Research Infrastructure The Netherlands (BBMRI-NL) complementation project CP2013-84-CP2013-83 and Dutch Scientific Research council NWO /ZonMW VICI scheme (nr. 91816631/528).

#### **STEMI\_GIPS-III**

The study received financial support from the Netherlands Organization for Medical Research (ZonMw; grant nr. 95103007); the funding source had no role in the study. GIPS-III 2010B257 The data are available upon request to all interested researchers provided their research question is within the informed consent provided by the participants. Patients did not provide informed consent to publicly release their data on an individual level on the internet. Data can be made available to other researchers upon request to Prof. Van der Harst after submitting a research proposal and approval of the GIPSIII steering committee responsible to ensure research question falls within the limits of the informed consent and IRB approval. The data will be released under a MTA to ensure future compliance to the obtained informed consent by other researchers.

#### **UCORBIO**

UCORBIO is conducted and supported by department of Cardiology, University Medical Center Utrecht, Netherlands. Folkert W. Asselbergs is supported by UCL Hospitals NIHR Biomedical Research Centre. Metabolic profiling was supported by Biobanking and BioMolecular resources Research Infrastructure, the Netherlands (BBMRI-NL). UCORBIO received funding from FP EU project CVgenes@target (HEALTH-F2-2013-601456). We would like to thank Ms. Jonne Hos and Ms. Merel Schurink for their logistical support and Daniel Kofink, PhD, for data management.

**VUNTR**

Funding was obtained from the Netherlands Organization for Scientific Research (NWO) and MagW/ZonMW grants 904-61-090, 985-10-002, 904-61-193, 480-04-004, 400-05-717, Addiction-31160008, Middelgroot-911-09-032, Spinozapremie 56-464-14192, Biobanking and Biomolecular Resources Research Infrastructure (BBMRI –NL, 184.021.007).; the European Community's Seventh Framework Program (FP7/2007-2013), ENGAGE (HEALTH-F4-2007-201413); the European Science Council (ERC Advanced, 230374), Rutgers University Cell and DNA Repository (NIMH U24 MH068457-06), the Avera Institute, Sioux Falls, South Dakota (USA) and the National Institutes of Health (NIH, R01D0042157-01A, MH081802, Grand Opportunity grants 1RC2 MH089951). We gratefully acknowledge grant NWO 480-15-001/674: Netherlands Twin Registry Repository: researching the interplay between genome and environment.

**HOF**

Main funding for initial data collection and analysis for the HOF study was provided by U.S National Institutes of Health grant R01-HL067914 and for subsequent analyses by grants R01-AG042190 and R01-AG066887.

**STABILITEIT**

## Ethics statements

### **Alpha Omega Cohort**

The protocol of the *Alpha Omega Trial*, from which the *Alpha Omega Cohort* emerged, was approved by the Medical Ethics Review Committee of Haga Hospital "Leyenburg", The Hague (METC Zuidwest Holland; L01.049) and by local ethics committees of all participating hospitals. In accordance with the Declaration of Helsinki, the *Alpha Omega Cohort* obtained informed consent from all participants prior to their entering the study.

### **Amsterdam Dementia Cohort**

The Amsterdam Dementia Cohort study protocol was approved by the Medical Ethics Committee of VU University Medical Centre (2016.061). In accordance with the Declaration of Helsinki, the Amsterdam Dementia Cohort obtained informed consent from all participants prior to their entering the study.

### **BIOMARCS**

The *BIOMarker study to identify the Acute risk of a Coronary Syndrome (BIOMArCS)* study protocol was approved by the *Medical Ethics Committee of the Erasmus MC (MEC-2007-185)*. In accordance with the Declaration of Helsinki, the *BIOMarker study to identify the Acute risk of a Coronary Syndrome (BIOMArCS)* obtained informed consent from all participants prior to their entering the study.

### **LUMINA**

The study protocols making up the *Leiden University Migraine Neuro-Analysis (LUMINA)* cohort were approved by the Medisch Ethische Toetsingcommissie (METC) of the Leiden University Medical Center (LUMC) (registration numbers P07.079 and P12.113). In accordance with the Declaration of Helsinki, the LUMINA CSF, MRS, and CHARM subcohorts obtained informed consent from all participants prior to their entering the study.

### **CHECK**

Cohort Heup en Cohort Knie study protocol was approved by Medical ethics committees of all participating centres. In accordance with the Declaration of Helsinki, the Cohort Heup en Cohort Knie obtained informed consent from all participants prior to their entering the study.

### **CODAM**

The Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) study protocol was approved by the Medical Ethics Review Committee of the AzM/UM (MEC 99-112 /MEC05-170). In accordance with the Declaration of Helsinki, the CODAM study obtained informed consent from all participants prior to their entering the study.

### **The Maastricht Study (TMS)**

The *Maastricht Study* protocol was approved by the Medical Ethics Review Committee AzM/UM (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (Permit 131088-105234-PG). In accordance with the Declaration of Helsinki, the *Maastricht Study* obtained informed consent from all participants prior to their entering the study.

### **The Hoorn Diabetes Care System cohort study (DCS)**

The Hoorn Diabetes Care System Cohort study protocol was approved by the ethical committee VU Vrije Universiteit Medical Center (2007/57). In accordance with the Declaration of Helsinki, the The Hoorn Diabetes Care System Cohort obtained informed consent from all participants prior to their entering the study.

### **Erasmus Rucphen Family (ERF) study**

The Erasmus Rucphen Family study protocol was approved by the Medical Ethics Committee of the Erasmus MC Rotterdam, the Netherlands (MEC 213.575/2002/114). In accordance with the Declaration of Helsinki, the Erasmus Rucphen Family study obtained informed consent from all participants prior to their entering the study.

#### **Rotterdam Study**

*The Rotterdam Study* protocol was approved by the Medical Ethics Committee of the Erasmus MC Rotterdam, the Netherlands. (MEC 02.1015) and by the Dutch Ministry of Health, Welfare, and Sport (Population Screening Act WBO, license number 1071272-159521-PG). In accordance with the Declaration of Helsinki, the *Rotterdam Study* obtained written informed consent from all participants prior to their entering the study.

#### **FUNCTGENOMICS**

*The 500 Functional Genomics* study protocol was approved by Commissie Mensgebonden Onderzoek Regio Arnhem-Nijmegen (42561.091.12). In accordance with the Declaration of Helsinki, the *500 Functional Genomics* study obtained informed consent from all participants prior to their entering the study.

#### **GARP**

The Genetics, ARthrosis and Progression study protocol was approved by the Medical Ethic Committee of the LUMC (P 76/98). In accordance with the Declaration of Helsinki, the Genetics, ARthrosis and Progression obtained informed consent from all participants prior to their entering the study.

#### **HELIUS**

*The HEalthy Lfe in an Uran Society (HELIUS)* study protocol was approved by the Medical Ethics Board of the Amsterdam University Medical Centers, location AMC (METC 10/100#10.17.1729). In accordance with the Declaration of Helsinki, the *HEalthy Lfe in an Uran Society* study obtained informed consent from all participants prior to their entering the study.

#### **LIFELINES-DEEP**

The Lifelines DEEP study was approved by the ethics committee of the University Medical Center Groningen, document number METC UMCG LLDEEP: M12.113965. All participants signed an informed consent form before study enrollment. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

#### **Leiden Longevity Study (LLS)**

The Leiden Longevity Study protocol was approved by the Medical Ethical Committee of the Leiden University Medical Center before the start of the study (P01.113). In accordance with the Declaration of Helsinki, the Leiden Longevity Study obtained informed consent from all participants prior to their entering the study.

#### **NESDA**

The Netherlands Study of Depression and Anxiety (NESDA) was approved by the Medical Ethical Committee of the VUmc (reference number 2003/183). In accordance with the Declaration of Helsinki, the Netherlands Study of Depression and Anxiety obtained informed consent from all participants prior to their entering the study.

#### **PROspective Study of Pravastatin in the Elderly at Risk (PROSPER)**

*The Prospective* study of Pravastatin in the Elderly at Risk protocol was approved by institutional ethics review boards of centres of Cork University (Ireland), Glasgow University (Scotland) and Leiden University Medical Center (the Netherlands). In accordance with the Declaration of Helsinki, the *The Prospective study of Pravastatin in the Elderly at Risk* obtained informed consent from all participants prior to their entering the study.

#### **The LUMC arthroplasty studies**

RAAK

Research Artrotisch Articulair Kraakbeen study protocol was approved by Medical Ethics Committee of the LUMC (P08.239 and P19.013). In accordance with the Declaration of Helsinki, the Research Artrotisch Articulair Kraakbeen obtained informed consent from all participants prior to their entering the study.

#### **TACTICS**

The 'Kwaliteit van leven en morbiditeit 10 jaar na totale heup of knie vervanging' study protocol was approved by Medical Ethics Committee of the LUMC (P00.179 and P11.050). In accordance with the Declaration of Helsinki, the Kwaliteit van leven en morbiditeit 10 jaar na totale heup of knie vervanging obtained informed consent from all participants prior to their entering the study.

#### **TOMAAT**

The '*Transfusie op maat studie - optimale bloedmanagement bij electieve orthopedische ingrepen*' study protocol was approved by Medical Ethics Committee of the LUMC (P03.044). In accordance with the Declaration of Helsinki, the Transfusie op maat studie - optimale bloedmanagement bij electieve orthopedische ingrepen obtained informed consent from all participants prior to their entering the study.

#### **STEMI\_GIPS-III**

The Glycometabolic Intervention as Adjunct to Primary Percutaneous Coronary Intervention in ST- Segment Elevation Myocardial Infarction (GIPS) III study is a double-blind placebo-controlled randomized clinical trial and was designed to determine whether metformin preserves left ventricular function after ST-segment elevation myocardial infarction (STEMI) in patients without diabetes. The study protocol was in accordance with the Declaration of Helsinki and was approved by the institutional review board (METC 2010.077, Groningen, the Netherlands) and national regulatory authorities. This trial was registered at clinicaltrials.gov (NCT01217307).

#### **UCORBIO**

Utrecht Coronary Biobank Study (UCORBIO) study protocol was approved by the Medical Ethics Committee of the UMC Utrecht (reference number 11–183). In accordance with the Declaration of Helsinki, the Utrecht Coronary Biobank Study (UCORBIO) obtained informed consent from all participants prior to their entering the study.

#### **VUNTR**

The Netherlands twin Register study protocol was approved by the Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Centre, Amsterdam, an Institutional Review Board certified by the U.S. Office of Human Research Protections (IRB number IRB00002991 under Federal-wide Assurance-FWA00017598). In accordance with the Declaration of Helsinki, the Netherlands Twin Register obtained informed consent from all participants prior to their entering the study.

#### **HOF**

The HOF study “Prenatal nutrition and adult disease, an analysis of sib-pairs discordant for exposure to the Dutch famine of 1944-1945” was approved by the Institutional Review Board of Columbia University, New York (IRB-AAAB4053) and by the Medical Ethics Committee of Leiden University Medical Center (P02.082). Written informed consent was obtained from all study participants.

#### **STABILITEIT**

The Amsterdam Dementia Cohort study protocol, from which the STABILITEIT cohort emerged, was approved by the Medical Ethics Committee of VU University Medical Centre (2016.061). In accordance with the Declaration of Helsinki, the Amsterdam Dementia Cohort obtained informed consent from all participants prior to their entering the study.