Psomagen Explore Training Report

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Contents

Overview

Psomagen performed an Explore training run on 2024-01-04. 88 replicates of pooled plasma samples were run on the Explore platform, panel(s) Cardiometabolic, Inflammation. The goal for this analysis was to report on certain sequencing and QC metrics, to perform preliminary exploratory analysis through PCA, to calculate the CV for each assay, and to perform an ANOVA to determine if there were any row or column effects. CV and ANOVA results are reported per assay and can be included in an Excel file and available upon request.

Sequencing Metrics

Table 1: Sequencing metrics.

Panel	Panel_Lot_Nr	reads	percentReadsPf
Cardiometabolic	B23407	638337024	69.57
Inflammation	E50006	638337024	69.98

PCA

To visualize the global data, scatterplots along the first two principal components were generated per panel and colored by both rows and columns (Figure 1). Appendix Figures 4 and 5 show additional PCA plots per block.

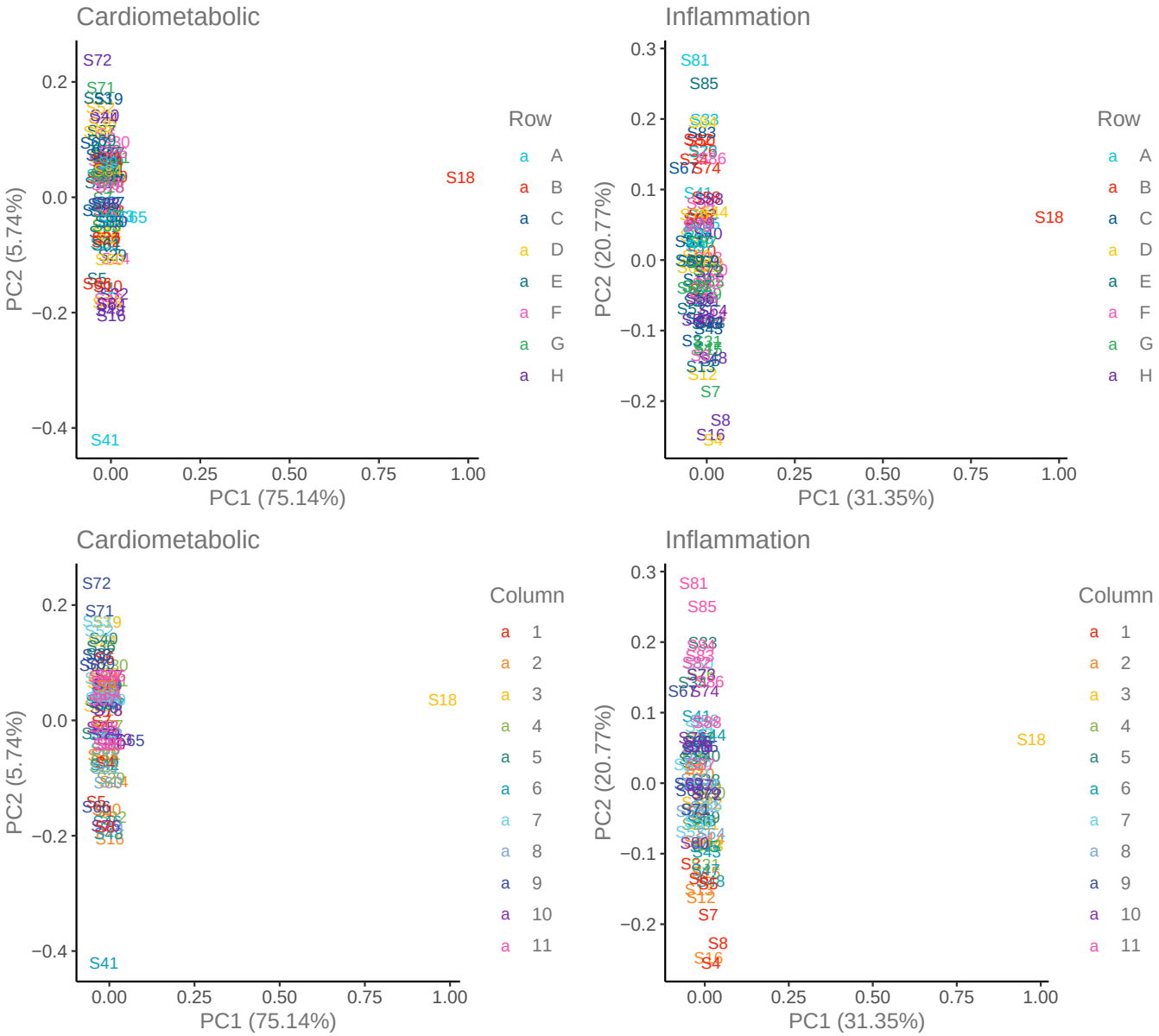

Figure 1: PCA plot of all samples plotted against the first two principal components for each panel, colored by either row or column.

Coefficient of variation

CV was calculated using the following formula:

$$CV = \sqrt{e^{\sigma^2} - 1} * 100$$
$$\sigma = ln(2) * \sigma_{Explore\ NPX}$$

Values under the limit of detection (LOD) were not used in the CV calculation. Table 2 shows the average CV per panel and block and also shows the distribution of assay CVs across different ranges. There were a total of 4 assays that did not have at least 2 sample NPX values above LOD to calculate CV. Figure 2 shows a histogram of all assay CVs. 76.8% of all assays had a CV less than 20%.

Table 2: Average CV per panel and block as well as count of assays with intra CV in different ranges of CV bins. NA indicates the number of assays that did not have enough samples with values above LOD to calculate CV for that many assays.

Panel	Block	Avg_CV	[0,5]	(5,10]	(10,15]	(15,25]	>25	NA
Cardiometabolic	A	18.8	0	3	27	22	16	1
	B	23.5	0	0	46	42	26	0
	C	18	0	44	35	12	16	0
	D	17.8	1	50	6	4	15	0
Inflammation	A	21.8	1	10	8	32	26	3
	B	14.8	1	11	51	39	4	0
	C	10.3	4	22	33	4	0	0
	D	17.9	0	20	70	10	14	0

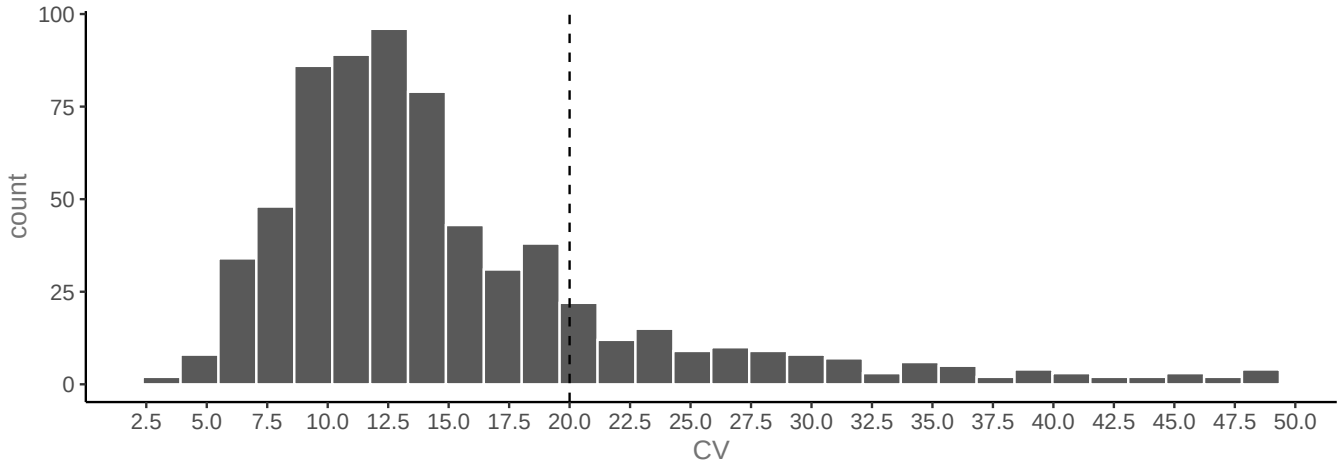

Figure 2: Histogram of CV values for all assays. Values below LOD were not used in CV calculations. Vertical dashed line indicates 20%. 76.8% of all assays have intra plate CVs less than 20%. 43 assays with CV greater than 50% are not included in this figure.

Inter-row and inter-column CV

Inter-row and inter-column CVs were also calculated. This was done per assay by calculating the CV of NPX values within each row or column and then averaging these CV estimates. Tables 3 and 4 shows the average row and column CVs, respectively, for each panel and block. Density curves are also shown in Figure 3.

Table 3: Average inter-row CVs per panel and block.

Panel	Block	CV
Cardiometabolic	A	17.4
	B	20.1
	C	18.7
	D	17.5
Inflammation	A	20.9
	B	12.8
	C	8.1
	D	18

Table 4: Average inter-column CVs per panel and block.

Panel	Block	CV
Cardiometabolic	A	18.3
	B	20.4
	C	16.2
	D	15.7
Inflammation	A	20.5
	B	13.8
	C	9.2
	D	16.1

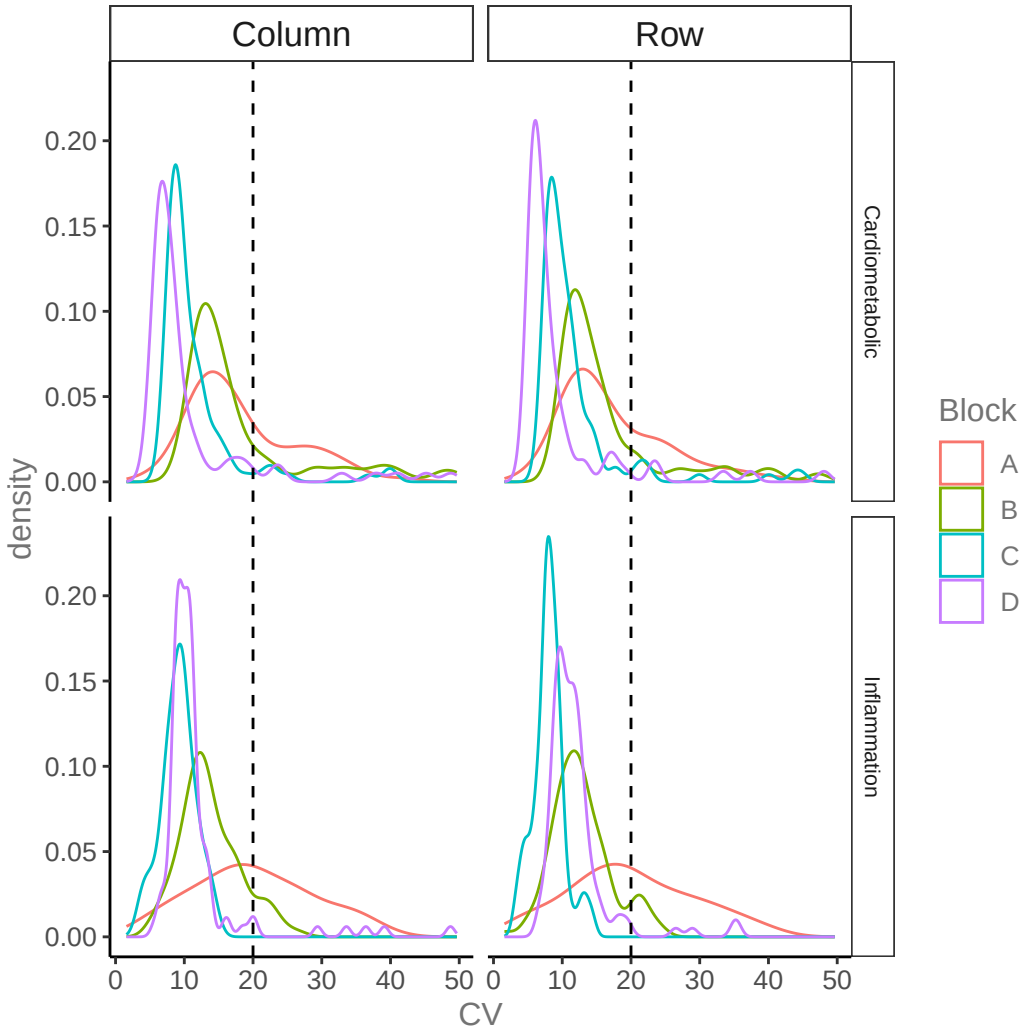

Figure 3: Distributions of inter-row and inter-column CVs. Note that 62 assays with CVs greater than 50% were removed from this figure.

Row and Column ANOVA

An ANOVA model was fit to each assay to determine if there are observable row and/or column effects. 169 of the 729 total assays were significant for either row effects, column effects or both. A total of 153 assays had a statistically significant column effect and a total of 104 assays had a statistically significant row effect (88 were significant for both row and column effects). Tables 5 and 6 summarizes this by panel and block.

Table 5: Count (percent) of assays with a statistically significant **row effect** effect in each panel and block.

Panel	Block	Count (percent) Significant Assays
Cardiometabolic	A	0 (0)
	B	1 (0.9)
	C	0 (0)
	D	0 (0)
Inflammation	A	12 (15)
	B	40 (37.7)
	C	51 (81)
	D	0 (0)

Table 6: Count (percent) of assays with a statistically significant **column effect** in each panel and block.

Panel	Block	Count (percent) Significant Assays
Cardiometabolic	A	16 (23.2)
	B	3 (2.6)
	C	0 (0)
	D	0 (0)
Inflammation	A	3 (3.8)
	B	73 (68.9)
	C	57 (90.5)
	D	1 (0.9)

Summary

No samples were excluded as outliers.

Appendix

PCA by panel and block

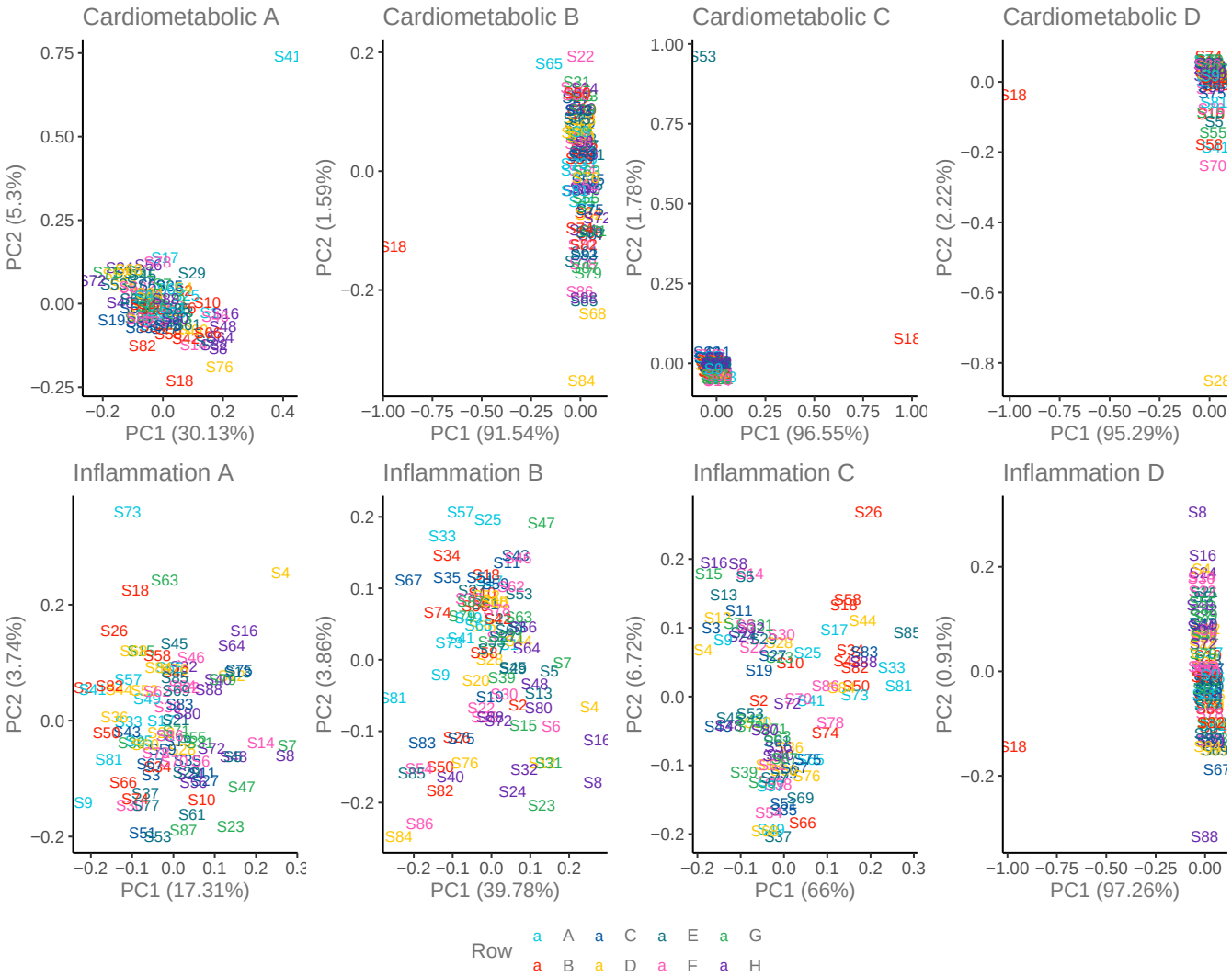

Figure 4: PCA of all samples by panel and block, colored by row.

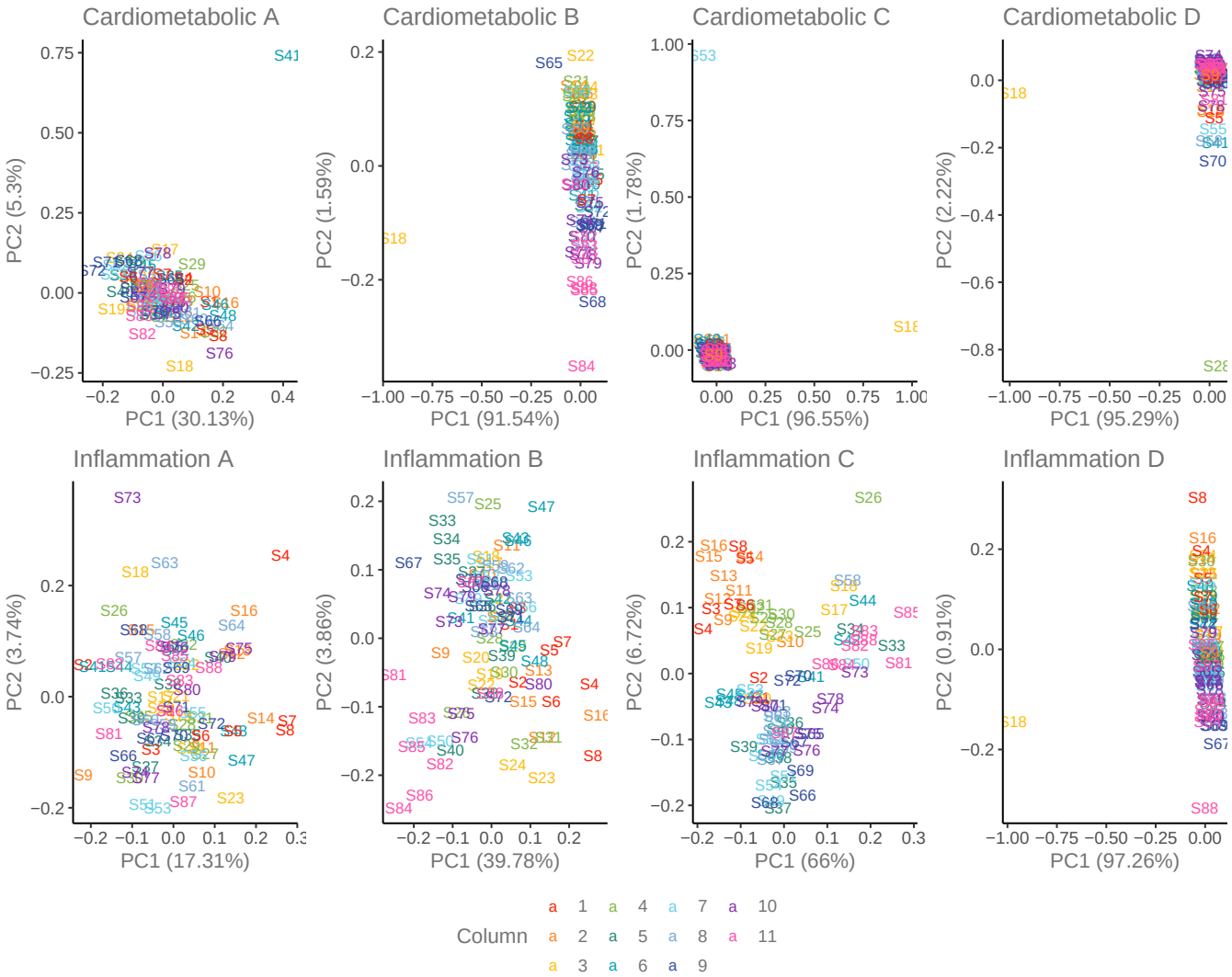

Figure 5: PCA of all samples by panel and block, colored by column.

Inter-row and inter-column CV

Table 7: Inter-row CVs

Panel	Row	Block A	Block B	Block C	Block D
Cardiometabolic	A	24.01	25.28	11.50	9.83
	B	20.47	69.89	63.83	74.31
	C	19.23	13.36	11.84	6.33
	D	20.87	15.22	9.14	16.61
	E	22.77	15.49	23.03	8.15
	F	21.41	11.80	11.12	9.71
	G	19.83	14.47	8.10	8.98
	H	25.08	15.41	8.09	9.77
Inflammation	A	27.06	14.83	11.34	9.43
	B	26.02	15.01	11.35	61.83
	C	25.95	16.39	9.47	13.14
	D	29.34	16.58	10.00	13.73
	E	27.86	16.09	10.82	13.32
	F	25.97	15.22	8.52	11.77
	G	27.28	15.48	7.30	12.85
	H	28.88	16.59	9.54	14.51

Table 8: Inter-column CVs

Panel	Column	Block A	Block B	Block C	Block D
Cardiometabolic	1	21.62	14.99	8.05	9.91
	2	21.44	11.09	9.90	12.79
	3	21.03	86.74	79.47	91.49
	4	22.13	10.82	10.69	19.64
	5	18.66	11.85	11.04	5.76
	6	26.19	10.65	10.86	10.04
	7	19.88	10.83	25.97	7.88
	8	20.61	13.24	10.81	9.00
	9	21.50	33.80	10.73	9.97
	10	21.09	15.89	9.91	7.54
	11	20.51	16.11	7.48	8.23
Inflammation	1	31.71	15.91	8.41	15.16
	2	32.64	16.19	8.89	10.84
	3	25.12	14.49	9.19	74.54
	4	24.49	14.37	8.77	12.91
	5	24.13	14.15	10.26	11.99
	6	28.81	15.71	11.48	11.35
	7	26.93	15.60	7.57	12.59
	8	30.74	13.51	10.94	13.71
	9	27.69	16.31	7.21	14.33
	10	25.58	14.47	8.20	12.07
	11	26.49	15.17	9.81	14.06

R Session Information

- R version 4.2.0 (2022-04-22), `x86_64-pc-linux-gnu`
- Locale: `LC_CTYPE=en_US.UTF-8`, `LC_NUMERIC=C`, `LC_TIME=en_US.UTF-8`, `LC_COLLATE=en_US.UTF-8`, `LC_MONETARY=en_US.UTF-8`, `LC_MESSAGES=en_US.UTF-8`, `LC_PAPER=en_US.UTF-8`, `LC_NAME=C`, `LC_ADDRESS=C`, `LC_TELEPHONE=C`, `LC_MEASUREMENT=en_US.UTF-8`, `LC_IDENTIFICATION=C`
- Running under: `Ubuntu 22.04.3 LTS`
- Matrix products: default
- BLAS: `/usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3`
- LAPACK: `/usr/lib/x86_64-linux-gnu/openblas-pthread/libopenblasp-r0.3.20.so`
- Base packages: base, datasets, graphics, grDevices, methods, stats, utils
- Other packages: dplyr 1.1.3, extrafont 0.19, forcats 1.0.0, ggplot2 3.4.4, ggpubr 0.6.0, kableExtra 1.3.4, knitr 1.45, lubridate 1.9.3, OlinkAnalyze 3.5.1, purrr 1.0.2, readr 2.1.4, stringr 1.5.0, tibble 3.2.1, tidyr 1.3.0, tidyverse 2.0.0
- Loaded via a namespace (and not attached): abind 1.4-5, backports 1.4.1, boot 1.3-28, broom 1.0.5, car 3.1-2, carData 3.0-5, cellranger 1.1.0, cli 3.6.1, coda 0.19-4, colorspace 2.1-0, compiler 4.2.0, cowplot 1.1.1, digest 0.6.33, emmeans 1.8.8, estimability 1.4.1, evaluate 0.22, extrafontdb 1.0, fansi 1.0.5, farver 2.1.1, fastmap 1.1.1, generics 0.1.3, ggrepel 0.9.3, ggsignif 0.6.4, glue 1.6.2, grid 4.2.0, gridExtra 2.3, gtable 0.3.4, hms 1.1.3, htmltools 0.5.6.1, httr 1.4.7, labeling 0.4.3, lattice 0.20-45, lifecycle 1.0.3, lme4 1.1-34, lmerTest 3.1-3, magrittr 2.0.3, MASS 7.3-56, Matrix 1.6-1.1, minqa 1.2.6, munsell 0.5.0, mvtnorm 1.2-3, nlme 3.1-157, nloptr 2.0.3, numDeriv 2016.8-1.1, pillar 1.9.0, pkgconfig 2.0.3, R6 2.5.1, Rcpp 1.0.11, readxl 1.4.3, rlang 1.1.1, rmarkdown 2.25, rstatix 0.7.2, rstudioapi 0.15.0, Rttf2pt1 1.3.12, rvest 1.0.3, scales 1.2.1, splines 4.2.0, stringi 1.8.1, svglite 2.1.2, systemfonts 1.0.5, tidyselect 1.2.0, timechange 0.2.0, tools 4.2.0, tzdb 0.4.0, utf8 1.2.3, vctrs 0.6.4, viridisLite 0.4.2, webshot 0.5.5, withr 2.5.1, xfun 0.40, xml2 1.3.5, yaml 2.3.7, zip 2.3.0