

# Long-Chain Acylcarnitines and Monounsaturated Fatty Acids Discriminate Heart Failure Patients According to Pulmonary Hypertension Status

## SUPPLEMENTARY MATERIAL

Table S1. Loading scores from the PCA analysis for the comparison noPH-HF vs controls and PH-HF vs controls.  
A. Comparison noPH-HF and controls

| Metabolites        | PC1 loading score | PC2 loading score |
|--------------------|-------------------|-------------------|
| <i>citrate</i>     | -0.198            | -0.192            |
| <i>glucose</i>     | -0.192            | -0.166            |
| <i>C18:1n7_FA</i>  | -0.184            | 0.027             |
| <i>C0_car</i>      | -0.175            | -0.075            |
| <i>TG</i>          | -0.163            | 0.181             |
| <i>pyruvate</i>    | -0.153            | -0.189            |
| <i>glycerol</i>    | -0.144            | -0.120            |
| <i>C18:1n9_FA</i>  | -0.133            | 0.146             |
| <i>C18:1_car</i>   | -0.127            | -0.135            |
| <i>C16:1n7_FA</i>  | -0.126            | 0.207             |
| <i>C3_car</i>      | -0.123            | -0.036            |
| <i>C16_car</i>     | -0.121            | -0.076            |
| <i>lactate</i>     | -0.120            | -0.258            |
| <i>C18_car</i>     | -0.108            | -0.079            |
| <i>succinate</i>   | -0.107            | -0.110            |
| <i>C22:1n9_FA</i>  | -0.104            | -0.081            |
| <i>C2_car</i>      | -0.099            | -0.094            |
| <i>C18:2_car</i>   | -0.091            | -0.084            |
| <i>C20:3n6_FA</i>  | -0.075            | 0.122             |
| <i>C20:3n9_FA</i>  | -0.073            | 0.133             |
| <i>C18:1n9T_FA</i> | -0.069            | 0.043             |
| <i>C22:4n6_FA</i>  | -0.066            | 0.165             |
| <i>insulin</i>     | -0.063            | -0.236            |
| <i>C18:0_FA</i>    | -0.061            | 0.040             |
| <i>C16:0_FA</i>    | -0.058            | 0.179             |

|                          |        |        |
|--------------------------|--------|--------|
| <i>C18:3n6G_FA</i>       | -0.057 | 0.265  |
| <i>C14:0_FA</i>          | -0.056 | 0.326  |
| <i>C5_car</i>            | -0.040 | -0.064 |
| <i>C12:0_FA</i>          | -0.032 | 0.118  |
| <i>C18:3n3a_FA</i>       | -0.024 | 0.152  |
| <i>C10:0_FA</i>          | -0.021 | 0.067  |
| <i>C14_car</i>           | -0.021 | 0.002  |
| <i>AcAc</i>              | 0.004  | -0.129 |
| <i>fumarate</i>          | 0.009  | -0.022 |
| <i>B-HB</i>              | 0.017  | -0.101 |
| <i>C20:1n9_FA</i>        | 0.031  | 0.133  |
| <i>C4_car</i>            | 0.045  | -0.017 |
| <i>FFA</i>               | 0.048  | 0.081  |
| <i>C22:5n3_FA</i>        | 0.056  | 0.170  |
| <i>C22:2n6_FA</i>        | 0.060  | 0.019  |
| <i>C20:3n3_FA</i>        | 0.065  | 0.250  |
| <i>C10_car</i>           | 0.071  | -0.077 |
| <i>C24:1n9_FA</i>        | 0.113  | -0.193 |
| <i>C20:2n6_FA</i>        | 0.117  | 0.048  |
| <i>total-cholesterol</i> | 0.159  | -0.042 |
| <i>C20:4n6_FA</i>        | 0.166  | 0.084  |
| <i>C16:1n7T_FA</i>       | 0.173  | 0.072  |
| <i>C20:0_FA</i>          | 0.191  | -0.158 |
| <i>LDL-cholesterol</i>   | 0.198  | -0.016 |
| <i>C18:2n6_FA</i>        | 0.205  | -0.185 |
| <i>C22:0_FA</i>          | 0.219  | -0.055 |
| <i>C24:0_FA</i>          | 0.269  | -0.030 |
| <i>HDL-cholesterol</i>   | 0.270  | 0.014  |
| <i>C22:6n3_FA</i>        | 0.281  | -0.098 |
| <i>C20:5n3_FA</i>        | 0.283  | 0.000  |

### *B. Comparison PH-HF and controls*

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| <b>Metabolites</b> | <b>PC1 loading score</b> | <b>PC2 loading score</b> |
|--------------------|--------------------------|--------------------------|
| <i>C18:1_car</i>   | -0.245                   | -0.141                   |

|                    |        |        |
|--------------------|--------|--------|
| <i>citrate</i>     | -0.214 | -0.166 |
| <i>C2_car</i>      | -0.204 | -0.074 |
| <i>C16_car</i>     | -0.192 | -0.090 |
| <i>C0_car</i>      | -0.189 | -0.030 |
| <i>C18:2_car</i>   | -0.186 | -0.043 |
| <i>C18:1n9_FA</i>  | -0.166 | 0.103  |
| <i>C22:4n6_FA</i>  | -0.148 | 0.171  |
| <i>C18:1n7_FA</i>  | -0.145 | 0.037  |
| <i>C18_car</i>     | -0.143 | -0.076 |
| <i>glucose</i>     | -0.139 | -0.144 |
| <i>fumarate</i>    | -0.078 | -0.115 |
| <i>C16:1n7_FA</i>  | -0.077 | 0.212  |
| <i>C3_car</i>      | -0.074 | 0.011  |
| <i>C14_car</i>     | -0.068 | 0.026  |
| <i>B-HB</i>        | -0.067 | -0.246 |
| <i>pyruvate</i>    | -0.065 | -0.055 |
| <i>C18:1n9T_FA</i> | -0.065 | -0.006 |
| <i>C20:3n9_FA</i>  | -0.064 | 0.271  |
| <i>C16:0_FA</i>    | -0.058 | 0.178  |
| <i>lactate</i>     | -0.056 | -0.122 |
| <i>AcAc</i>        | -0.054 | -0.250 |
| <i>C12:0_FA</i>    | -0.046 | 0.094  |
| <i>glycerol</i>    | -0.045 | -0.111 |
| <i>C14:0_FA</i>    | -0.032 | 0.293  |
| <i>C22:1n9_FA</i>  | -0.030 | 0.072  |
| <i>TG</i>          | -0.026 | 0.160  |
| <i>C18:0_FA</i>    | -0.026 | -0.009 |
| <i>succinate</i>   | -0.025 | -0.134 |
| <i>C10:0_FA</i>    | -0.022 | 0.048  |
| <i>C20:1n9_FA</i>  | -0.021 | 0.112  |
| <i>C18:3n6G_FA</i> | -0.009 | 0.309  |
| <i>C22:2n6_FA</i>  | -0.002 | -0.014 |
| <i>C5_car</i>      | 0.021  | 0.105  |
| <i>C4_car</i>      | 0.031  | 0.034  |
| <i>C20:3n6_FA</i>  | 0.038  | 0.161  |

|                          |       |        |
|--------------------------|-------|--------|
| <i>insulin</i>           | 0.039 | -0.004 |
| <i>C18:3n3_FA</i>        | 0.055 | 0.095  |
| <i>C10_car</i>           | 0.059 | -0.062 |
| <i>C22:5n3_FA</i>        | 0.068 | 0.244  |
| <i>C16:1n7T_FA</i>       | 0.072 | -0.037 |
| <i>C20:4n6_FA</i>        | 0.080 | 0.047  |
| <i>C24:1n9_FA</i>        | 0.081 | -0.210 |
| <i>FFA</i>               | 0.091 | -0.067 |
| <i>C20:3n3_FA</i>        | 0.145 | 0.182  |
| <i>C20:0_FA</i>          | 0.159 | -0.162 |
| <i>C20:2n6_FA</i>        | 0.163 | -0.031 |
| <i>C18:2n6_FA</i>        | 0.191 | -0.239 |
| <i>C22:0_FA</i>          | 0.199 | -0.054 |
| <i>C20:5n3_FA</i>        | 0.224 | 0.070  |
| <i>C22:6n3_FA</i>        | 0.230 | -0.050 |
| <i>C24:0_FA</i>          | 0.245 | -0.069 |
| <i>LDL-cholesterol</i>   | 0.265 | -0.036 |
| <i>HDL-cholesterol</i>   | 0.270 | 0.009  |
| <i>total-cholesterol</i> | 0.284 | -0.035 |

In grey, the top 15 metabolites with the higher relative score plot

**Table S2. Interaction analysis with statins treatment**

| <b>Statins</b>               |                |              |
|------------------------------|----------------|--------------|
|                              | <i>noPH-HF</i> | <i>PH-HF</i> |
| <b>Acylcarnitines</b>        |                |              |
| <b>free carnitine</b>        | NS             | NS           |
| <b>C2-AC</b>                 | NS             | NS           |
| <b>C16-AC</b>                | NS             | NS           |
| <b>C18:1-AC</b>              | NS             | NS           |
| <b>C18:2-AC</b>              | NS             | NS           |
| <b>Saturated fatty acids</b> |                |              |
| <b>C20:0</b>                 | NS             | NS           |

|                                    |    |    |
|------------------------------------|----|----|
| <b>C22:0</b>                       | NS | NS |
| <b>C24:0</b>                       | NS | NS |
| <b>Monounsaturated fatty acids</b> |    |    |
| <b>C16:1n7</b>                     | NS | NS |
| <b>C18:1n7</b>                     | NS | NS |
| <b>C18:1n9</b>                     | NS | NS |
| <b>Polyunsaturated fatty acids</b> |    |    |
| <b>C18:2n6</b>                     | NS | NS |
| <b>C20:4n6</b>                     | NS | NS |
| <b>C20:5n3</b>                     | NS | NS |
| <b>C22:4n6</b>                     | NS | NS |
| <b>C22:5n3</b>                     | NS | NS |
| <b>C22:6n3</b>                     | NS | NS |

In the presence of significant interaction, the correlation between metabolite and NT-proBNP was tested in each group and the corresponding *P* values were noted. NS: not significant.

**Table S3. Interaction analysis with diuretics treatment**

| <b>Diuretics</b>             |         |       |
|------------------------------|---------|-------|
|                              | noPH-HF | PH-HF |
| <b>Acylcarnitines</b>        |         |       |
| <b>free carnitine</b>        | NS      | NS    |
| <b>C2-AC</b>                 | NS      | NS    |
| <b>C16-AC</b>                | NS      | NS    |
| <b>C18:1-AC</b>              | NS      | NS    |
| <b>C18:2-AC</b>              | <0.05   | NS    |
| <b>Saturated fatty acids</b> |         |       |
| <b>C20:0</b>                 | NS      | NS    |

|                                    |    |    |
|------------------------------------|----|----|
| <b>C22:0</b>                       | NS | NS |
| <b>C24:0</b>                       | NS | NS |
| <b>Monounsaturated fatty acids</b> |    |    |
| <b>C16:1n7</b>                     | NS | NS |
| <b>C18:1n7</b>                     | NS | NS |
| <b>C18:1n9</b>                     | NS | NS |
| <b>Polyunsaturated fatty acids</b> |    |    |
| <b>C18:2n6</b>                     | NS | NS |
| <b>C20:4n6</b>                     | NS | NS |
| <b>C20:5n3</b>                     | NS | NS |
| <b>C22:4n6</b>                     | NS | NS |
| <b>C22:5n3</b>                     | NS | NS |
| <b>C22:6n3</b>                     | NS | NS |

In the presence of significant interaction, the correlation between metabolite and NT-proBNP was tested in each group and the corresponding *P* values were noted. NS: not significant.

**Table S4. Interaction analysis with diabetic (type 2 diabetes) status**

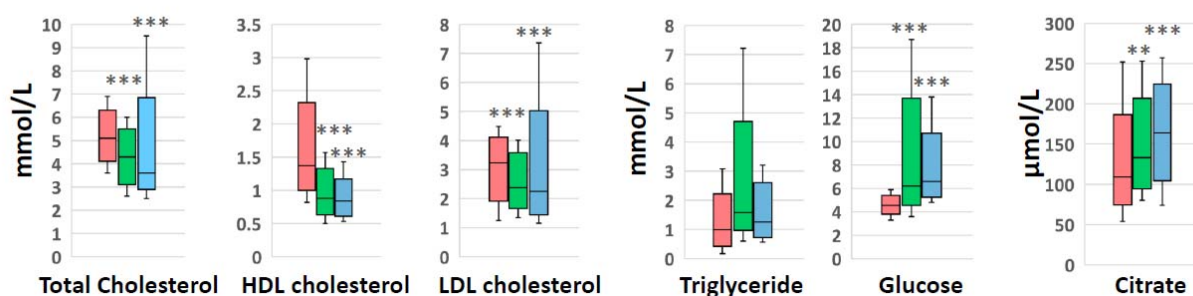
| <b>Diabetes</b>              |         |       |
|------------------------------|---------|-------|
|                              | noPH-HF | PH-HF |
| <b>Acylcarnitines</b>        |         |       |
| <b>free carnitine</b>        | NS      | NS    |
| <b>C2-AC</b>                 | NS      | NS    |
| <b>C16-AC</b>                | NS      | NS    |
| <b>C18:1-AC</b>              | NS      | NS    |
| <b>C18:2-AC</b>              | NS      | NS    |
| <b>Saturated fatty acids</b> |         |       |
| <b>C20:0</b>                 | NS      | NS    |
| <b>C22:0</b>                 | NS      | NS    |

|                                    |    |    |
|------------------------------------|----|----|
| <b>C24:0</b>                       | NS | NS |
| <b>Monounsaturated fatty acids</b> |    |    |
| <b>C16:1n7</b>                     | NS | NS |
| <b>C18:1n7</b>                     | NS | NS |
| <b>C18:1n9</b>                     | NS | NS |
| <b>Polyunsaturated fatty acids</b> |    |    |
| <b>C18:2n6</b>                     | NS | NS |
| <b>C20:4n6</b>                     | NS | NS |
| <b>C20:5n3</b>                     | NS | NS |
| <b>C22:4n6</b>                     | NS | NS |
| <b>C22:5n3</b>                     | NS | NS |
| <b>C22:6n3</b>                     | NS | NS |

In the presence of significant interaction, the correlation between metabolite and NT-proBNP was tested in each group and the corresponding *P* values were noted. NS: not significant.

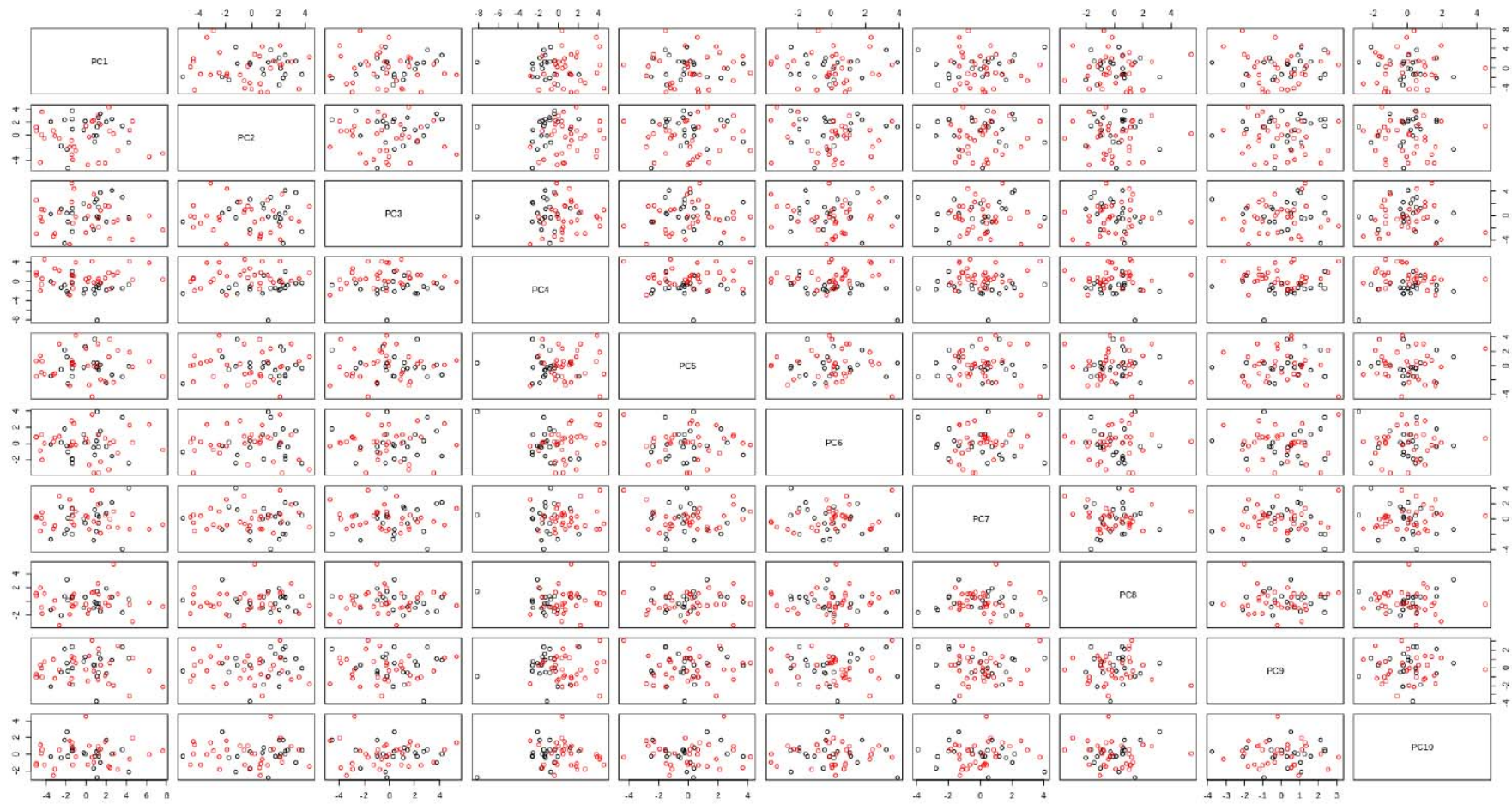
### A . Other metabolites

● controls ● noHF-PH ● PH-HF



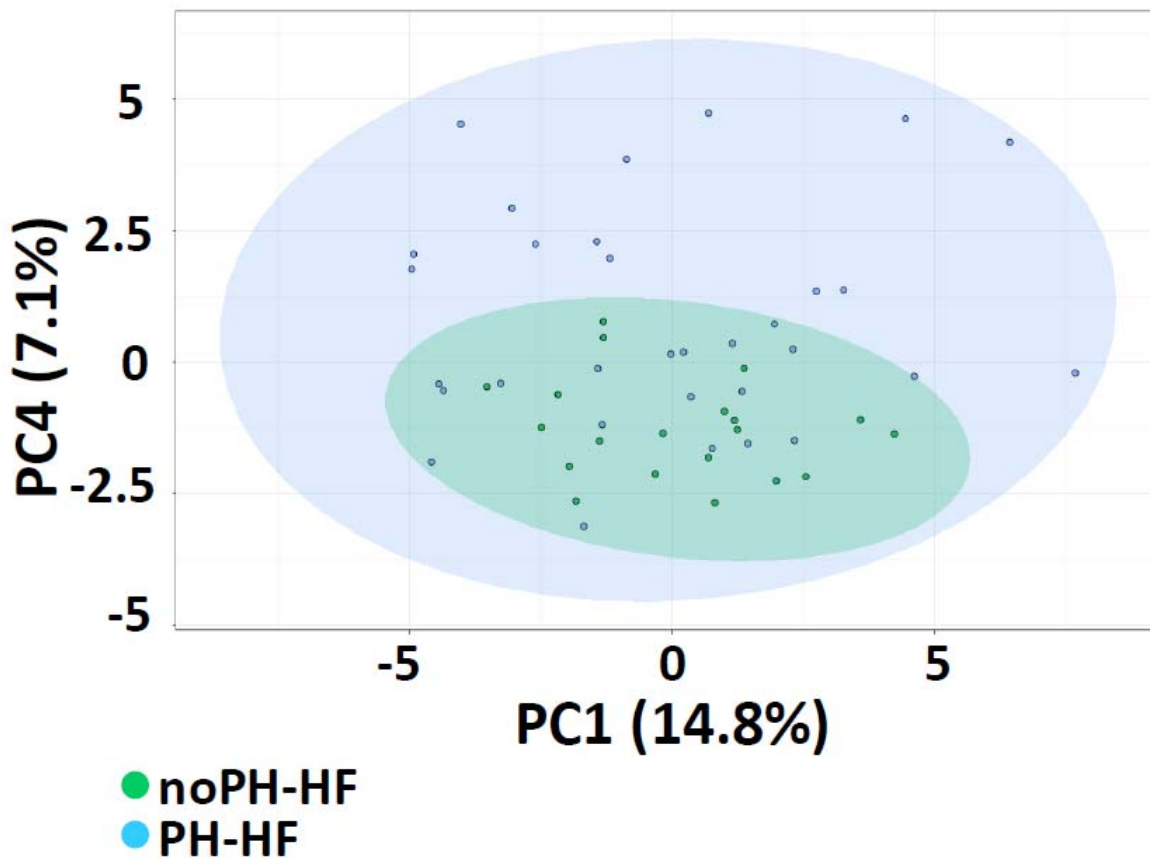
**Figure S1. Other metabolites that are differentially changed in PH-HF (N=33) and noPH-HF (N=27) compared to controls (N=72).**

Box plots depicting the most discriminant molecules from commercial biochemical analyses and citrate, which was measured using a MS-based approach. Comparison of noPH-HF (green) vs. controls (red) and PH-HF (blue) vs. controls. In the boxplots, rectangles represent the SD, the segment inside the rectangle the median and the whiskers above and below the maximum and minimum. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001 compared to controls.



**Figure S2. PCA analysis reporting all the PC combination.** This whole PCA analysis was used to identify the better association and we observed a potential structure only in the presence of the PC4.





**Figure S3. Principal component analysis identified a higher heterogeneity in PH-HF (N=33) compared to noPH-HF (N=27).** A total of 55 variables were included in the analysis and comprised usual biochemical parameters as well as various metabolites measured by a combination of MS-based metabolomics approach targeting fatty acids, acyl-carnitines, organic acids and amino acids. Principal component 1 (PC1) and principal component 4 (PC4) accounted for 14.8% and 7.1% of the total variation, respectively. PH-HF were identified in blue and noPH-HF in green. Note that we excluded one outlier sample that was outside the confident ellipse.

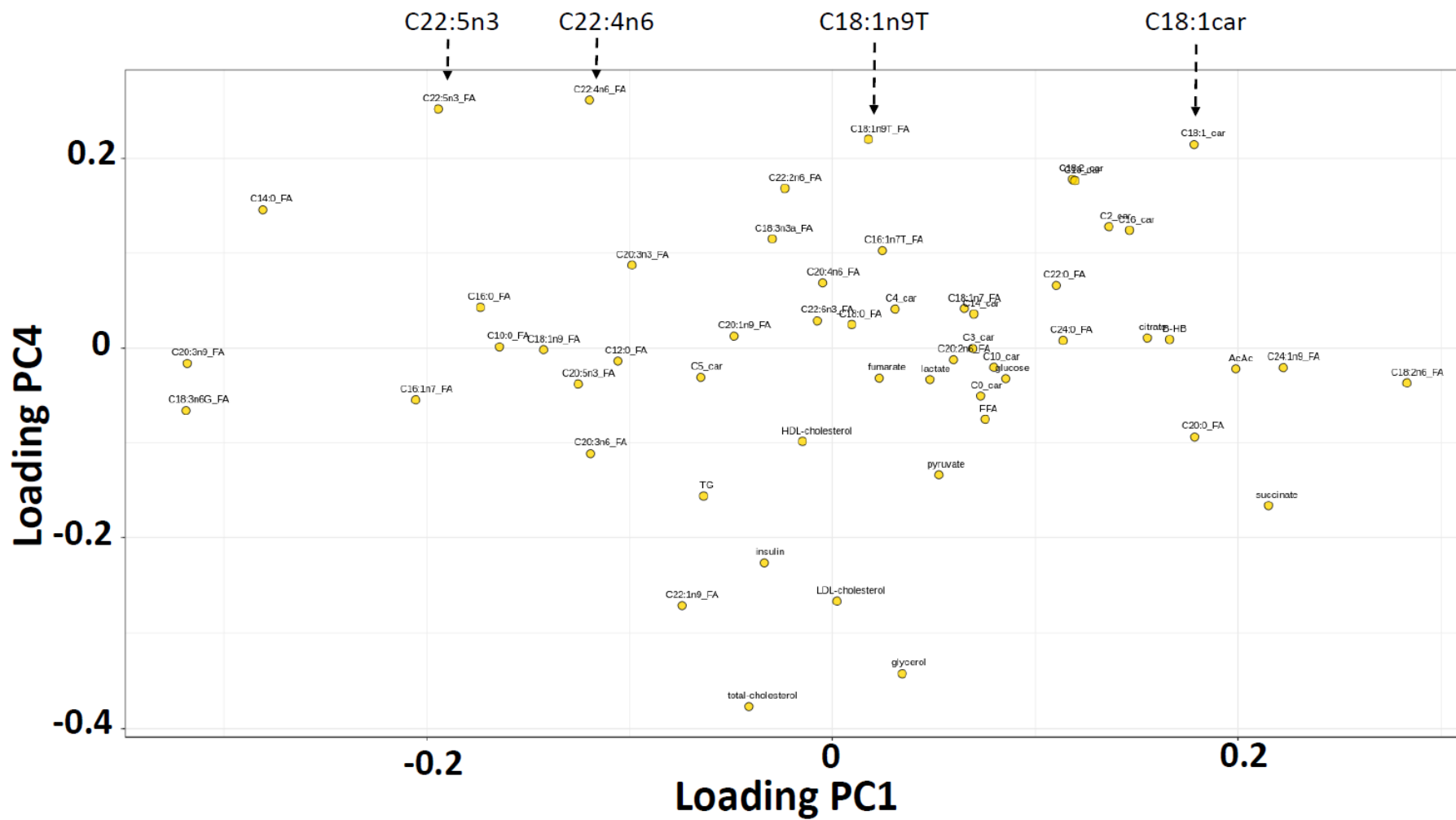
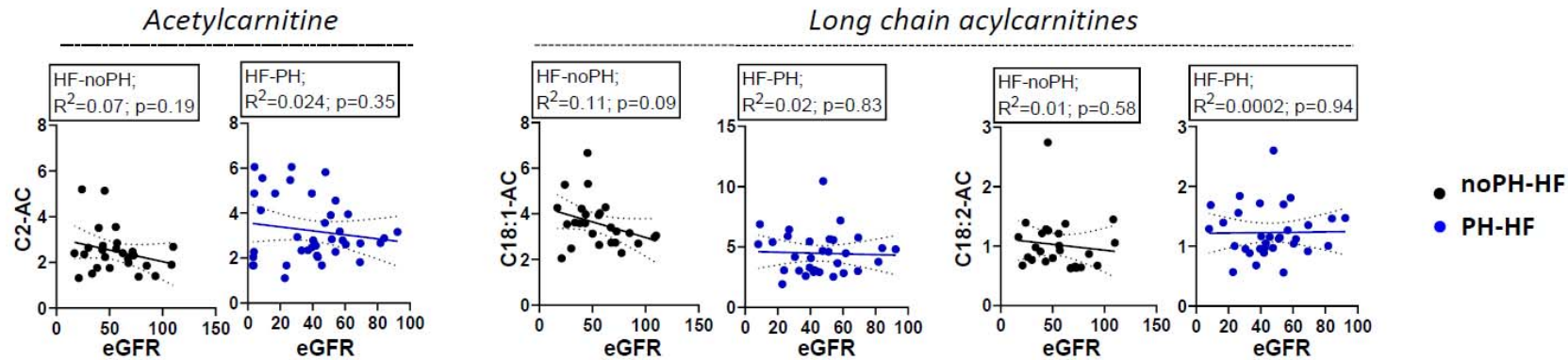


Figure S4. Loading plot analysis showing the metabolites used for the PCA analysis. We highlighted the 4 metabolites participating the most to the heterogeneity of the PH-HF group

## A . Acylcarnitines



## B. Fatty acids

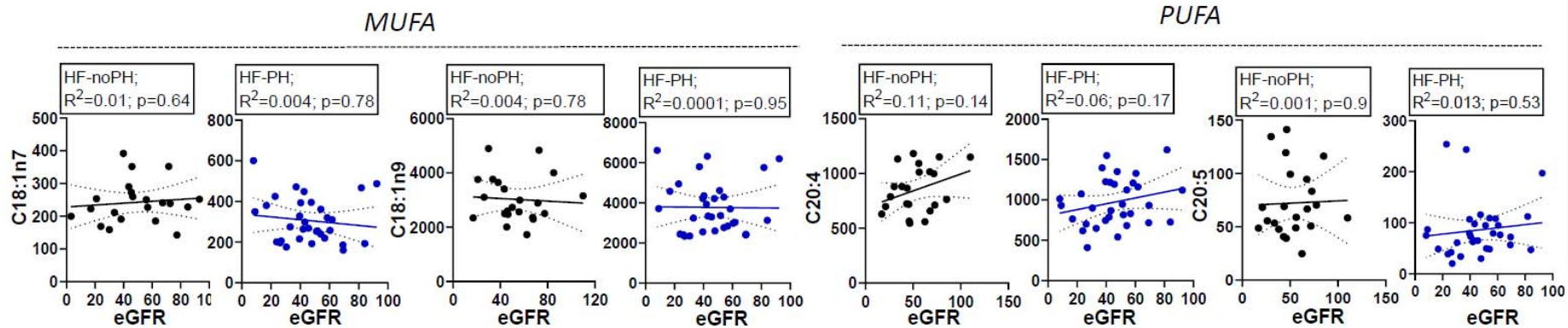


Figure S5. Acylcarnitines and fatty acids do not correlate with eGFR in PH-HF and noPH-HF. Pearson correlation analysis between eGFR and (A) acylcarnitines, (B) FA (MUFA and PUFA) in noPH-HF (black) or PH-HF (blue) patients. The dotted lines indicate the 95% confidence intervals.

