Mild heat induces a distinct “eustress” response in Chinese Hamster Ovary cells but does not induce heat shock protein synthesis

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**Supplementary Figure S1: The effect of HS duration on HSP induction.** A GPI-mGFP–expressing CHO cell line was subjected to heat treatments at 40°C (A) and 42.5°C (B) for the specified time periods, and the samples were prepared for western blotting after 6 h of recovery at 37°C. Full lengths blots are presented in Supplementary Figure S7 and S8 for Figure S1A and S1B, respectively.
**Supplementary Figure S2: The effect of heat treatment on the phosphorylation status of HSP25.** CHO cells harboring the GPI-mGFP construct were incubated at a given temperature for 20 min before sample preparation for western blotting. See Supplementary Figure S9 for full length blots.
Supplementary Figure S3: HSP25 distribution after a second heat treatment of MEF cells. Representative images of HSP25 distribution in MEF cells at 37°C; after a 20 min heat treatment at specified temperatures (1st heat); followed by 6 h recovery at 37°C (rec 37°C); and after a second, 20 min, heat treatment at specified temperatures (2nd heat).
Supplementary Figure S4: The alteration of membrane diffusion of Bodipy FL-SM probe in live cells at different heat exposures. Changes in the diffusion coefficient (D) and confinement time ($\tau_0$) of Bodipy FL-SM on the surface of wild-type CHO cells were measured by ImFCS. The values represent averages, and the error bars represent the standard error of mean (SEM) (n=9, p<0.05).
Supplementary Figure S5: Full Western blots of Figure 1A. The boxed image depicts what is shown in figure 1A. Blots were carried out on a single membrane with the following order of primary antibodies; HSP25, HSP70, HSP60 - GAPDH, HSP90 and GRP78. Treatments at 37°C (1), 40°C (2), 42.5°C (3) and 44°C (4) were shown in triplicates (A, B and C).
Supplementary Figure S6: Full Western blots of Figure 1B. The boxed image depicts what is shown in figure 1B. Treatments at 37°C (1), 40°C (2), 42.5°C (3) and 44°C (4) were shown in triplicates (A, B and C).
Supplementary Figure S7: Full Western blots of Supplementary Figure S1A. The boxed image depicts what is shown in Supplementary Figure S1A. Blots were carried out on a single membrane with the following order of primary antibodies: HSP25, HSP70, HSP60 - GAPDH, HSP90 and GRP78. Treatments at 0 min (1), 10 min (2), 20 min (3), 40 min (4) and 60 min (5) were shown in duplicates (A and B).
Supplementary Figure S8: Full Western blots of Supplementary Figure S1B. The boxed image depicts what is shown in Supplementary Figure S1B. Blots were carried out on a single membrane with the following order of primary antibodies; HSP25, HSP70, HSP60 - GAPDH, HSP90 and GRP78. Treatments at 0 min (1), 10 min (2), 20 min (3), 40 min (4) and 60 min (5) were shown in duplicates (A and B).
Supplementary Figure S9: Full Western blots of Supplementary Figure S2. The boxed image depicts what is shown in supplementary figure S2. Treatments at 37°C (1), 40°C (2), 42.5°C (3) and 44°C (4) were shown in triplicates (A, B and C).
Supplementary methods

ImFCS data analysis

The autocorrelation functions (ACFs) for every pixel were calculated using a multi-tau correlation scheme. An exponential of polynomial bleach correction was used to correct data before fitting. To obtain the diffusion coefficient (D) for all pixels ACFs were fitted according to the equation below.

\[ G(\tau) = \frac{1}{N} + \left[ \frac{\text{erf}(p(\tau)) + \left( e^{-\frac{(p(\tau))^2}{\pi p(\tau)}} - 1 \right)}{\sqrt{\pi p(\tau)}} \right]^2 + G_\infty p(\tau) = \frac{a}{\sqrt{4D\tau + \omega_0^2}} \]

Where \( G(\tau) \) is the ACF, \( \tau \) is the correlation time, \( N \) is the number of detected particles, \( a \) is the pixel size, and \( \omega_0 \) is the \( 1/e^2 \) radius of the Gaussian approximation of the point spread function. To identify and describe the mode of membrane organization by investigating the size-dependency of diffusion coefficient, we used the Imaging FCS type of FCS diffusion law. According to that, the diffusion time (\( \tau_D \)) of the fluorescent probe depends on the observation area \( (A_{eff}) \), as described by

\[ \tau_D(A_{eff}) = \tau_0 + \frac{A_{eff}}{D} \]

where \( A_{eff} \) is the area of the membrane in which the labeled particle travels across, and is calculated by the convolution of the detection area with the point spread function. \( \tau_0 \) is the intercept of the diffusion law plot on the y-axis of \( A_{eff}/D \) vs. \( A_{eff} \). This parameter provides information about the diffusion confinement. The diffusion law can be plotted by using different \( A_{eff} \) values that are calculated by post-acquisition binning of pixels. In the case of free diffusion, \( D \) is constant regardless of \( A_{eff} \) so \( \tau_D(A_{eff}) \) is a straight line passing through the origin \( (\pm 0.1 \text{ s}) \) in the diffusion law graph. A heterogeneous system, where membrane domains or meshwork are present, however, allows spatial scale-dependent diffusion, which results in a remarkably different diffusion law plot with positive or negative intercepts for domain partitioning or meshwork diffusion, respectively.

Lipidomics methods

Lipidomics analyses were performed on an LTQ-Orbitrap Elite instrument (Thermo Fisher Scientific, Bremen, Germany) equipped with a robotic nanoflow ion source TriVersa NanoMate (Advion BioSciences, Ithaca, NY), using chips with 5.5-µm diameter spraying nozzles. The ion source was controlled by Chipsoft 8.3.1 software (Advion). The ionization voltages were +1.3 kV and −1.9 kV in the positive and negative modes, respectively, and the backpressure was set at 1 psi in both modes. The temperature of the ion transfer capillary was 330°C. Data acquisition was performed at the mass resolution R_m/z,400=240,000.

Phosphatidylcholine (PC, diacyl, and PC-O, alkyl-acyl), lysophosphatidylcholine (LPC), and sphingomyelin (SM) were detected and quantified in the positive ion mode; phosphatidylethanolamine (PE, diacyl, and PE-Pl, alkenyl-acyl), lysophosphatidylethanolamine (LPE), phosphatidylinositol (PI), lysophosphatidylinositol (LPI), phosphatidylserine (PS), phosphatidic acid (PA), phosphatidylglycerol
(PG), cardiolipin (CL), ceramide (Cer), glucosyliceramide (GlcCer), and ganglioside (GM3) were detected and quantified in the negative ion mode.

For quantification, 10 µL of the lipid extract was diluted with 140 µL of the infusion solvent mixture (chloroform:methanol:iso-propanol, 1:2:1, by vol.) containing an internal standard mix (71 pmol PC d31-16:0/18:1, 25 pmol PE d31-16:0/18:1, 11 pmol PI d31-16:0/18:1, 19 pmol PS d31-16:0/18:1, 2.5 pmol PG d31-16:0/18:1, 1 pmol PA d31-16:0/18:1, 1.5 pmol CL 56:0, 5 pmol SM d18:1/17:0, 2 pmol Cer d18:1/17:0, 3 pmol GlCer d18:1/12:0 and 5 pmol GM3 d3-d18:1/18:0). Next, the mixture was split in two, and 5% (final concentration) dimethylformamide (additive for the negative ion mode) or 3 mM (final concentration) ammonium chloride (additive for the positive ion mode) were added to the sample halves. Each quantified lipid species accounted for more than 0.5% of the respective lipid class. The mass tolerance was 3 ppm. Data files generated by LipidXplorer queries were further processed by in-house Excel macros.

The sum of absolute mol% difference relative to control (SoamD score) values were calculated based on Tarasov et al, (2014), as follows:

\[ \text{SoamD} = \Sigma \text{abs(mol\%}_i, T - \text{mol\%}_i, 37) \]

where \( \text{mol\%}_i, T \) is the mol% of membrane lipids value for lipid species i at stress temperature T, and \( \text{mol\%}_i, 37 \) is the mol% of membrane lipids value for lipid species i at control temperature (37°C).
**Supplementary Table S1. Detailed lipidomic dataset**

*Data are expressed as mol% of membrane lipids and given as mean±SD, n=4;*  
*p<0.05, **p<0.01 (vs. 37 °C)*

| Name | 37 °C  | 40 °C     | 42.5 °C    | 44 °C     |
|------|--------|-----------|------------|-----------|
| LPC [16:0] | 0.356 ± 0.023 | 0.269 ± 0.039 ** | 0.168 ± 0.019 ** | 0.134 ± 0.020 ** |
| LPC [18:1] | 0.119 ± 0.034 | 0.074 ± 0.037 | 0.041 ± 0.022 ** | 0.030 ± 0.009 ** |
| LPC [18:0] | 0.132 ± 0.017 | 0.096 ± 0.014 * | 0.064 ± 0.012 ** | 0.059 ± 0.008 ** |
| LPC | 0.606 ± 0.059 | 0.439 ± 0.062 ** | 0.273 ± 0.035 ** | 0.222 ± 0.030 ** |
| PC [28:0] | 0.177 ± 0.025 | 0.161 ± 0.015 | 0.147 ± 0.008 | 0.152 ± 0.015 |
| PC [30:1] | 0.325 ± 0.010 | 0.310 ± 0.006 * | 0.315 ± 0.014 | 0.298 ± 0.019 * |
| PC [30:0] | 1.853 ± 0.210 | 1.818 ± 0.097 | 1.781 ± 0.123 | 1.692 ± 0.129 |
| PC [32:2] | 0.360 ± 0.016 | 0.353 ± 0.018 | 0.350 ± 0.015 | 0.333 ± 0.014 * |
| PC [32:1] | 4.664 ± 0.086 | 4.561 ± 0.075 | 4.392 ± 0.124 * | 4.429 ± 0.065 ** |
| PC [32:0] | 3.372 ± 0.226 | 3.395 ± 0.294 | 3.554 ± 0.299 | 3.334 ± 0.212 |
| PC [34:3] | 0.338 ± 0.027 | 0.320 ± 0.014 | 0.321 ± 0.015 | 0.315 ± 0.023 |
| PC [34:2] | 4.097 ± 0.063 | 3.917 ± 0.060 ** | 3.877 ± 0.108 * | 3.890 ± 0.109 * |
| PC [34:1] | 19.814 ± 0.724 | 19.178 ± 0.309 | 18.993 ± 0.600 | 18.518 ± 0.407 * |
| PC [34:0] | 0.380 ± 0.111 | 0.309 ± 0.043 | 0.330 ± 0.044 | 0.311 ± 0.038 |
| PC [36:5] | 0.401 ± 0.031 | 0.384 ± 0.016 | 0.371 ± 0.013 | 0.353 ± 0.016 * |
| PC [36:4] | 2.160 ± 0.241 | 2.042 ± 0.068 | 1.993 ± 0.085 | 1.875 ± 0.092 |
| PC [36:3] | 1.688 ± 0.042 | 1.591 ± 0.034 * | 1.574 ± 0.051 * | 1.549 ± 0.057 ** |
| PC [36:2] | 7.242 ± 0.250 | 6.889 ± 0.185 | 6.824 ± 0.199 * | 6.847 ± 0.180 * |
| PC [36:1] | 1.826 ± 0.075 | 1.755 ± 0.049 | 1.730 ± 0.049 | 1.736 ± 0.041 |
| PC [38:6] | 1.168 ± 0.089 | 1.085 ± 0.031 | 1.089 ± 0.050 | 0.997 ± 0.054 * |
| PC [38:5] | 1.640 ± 0.110 | 1.549 ± 0.039 | 1.540 ± 0.071 | 1.434 ± 0.078 * |
| PC [38:4] | 1.022 ± 0.110 | 0.953 ± 0.056 | 0.958 ± 0.047 | 0.888 ± 0.065 |
|        |        |        |        |        |
|--------|--------|--------|--------|--------|
| PC [38:3] | 0.425 ± 0.032 | 0.389 ± 0.011 | 0.400 ± 0.024 | 0.366 ± 0.016 * |
| PC [38:2] | 0.486 ± 0.010 | 0.461 ± 0.010 * | 0.466 ± 0.023 | 0.458 ± 0.009 ** |
| PC [40:7] | 0.439 ± 0.010 | 0.418 ± 0.008 * | 0.416 ± 0.012 * | 0.381 ± 0.008 ** |
| PC [40:6] | 0.514 ± 0.040 | 0.492 ± 0.023 | 0.499 ± 0.027 | 0.452 ± 0.032 |
| PC [40:5] | 0.349 ± 0.013 | 0.330 ± 0.008 * | 0.341 ± 0.022 | 0.318 ± 0.029 |
| **PC** | 54.741 ± 2.105 | 52.659 ± 0.916 | 52.261 ± 1.764 | 50.925 ± 1.162 * |
| PC-O [30:0] | 0.149 ± 0.009 | 0.156 ± 0.015 | 0.154 ± 0.020 | 0.167 ± 0.025 |
| PC-O [32:1] | 0.597 ± 0.021 | 0.576 ± 0.048 | 0.594 ± 0.060 | 0.612 ± 0.053 |
| PC-O [32:0] | 0.542 ± 0.046 | 0.501 ± 0.057 | 0.542 ± 0.096 | 0.567 ± 0.065 |
| PC-O [34:2] | 0.540 ± 0.065 | 0.564 ± 0.036 | 0.576 ± 0.058 | 0.585 ± 0.046 |
| PC-O [34:1] | 3.431 ± 0.115 | 3.326 ± 0.181 | 3.446 ± 0.177 | 3.591 ± 0.182 |
| PC-O [34:0] | 0.132 ± 0.013 | 0.137 ± 0.012 | 0.164 ± 0.024 | 0.170 ± 0.025 * |
| PC-O [36:6] | 0.014 ± 0.011 | 0.026 ± 0.013 | 0.030 ± 0.003 * | 0.032 ± 0.005 * |
| PC-O [36:5] | 0.252 ± 0.092 | 0.299 ± 0.013 | 0.316 ± 0.029 | 0.303 ± 0.014 |
| PC-O [36:4] | 1.237 ± 0.069 | 1.158 ± 0.062 | 1.185 ± 0.131 | 1.164 ± 0.076 |
| PC-O [36:3] | 0.365 ± 0.056 | 0.371 ± 0.009 | 0.375 ± 0.025 | 0.389 ± 0.018 |
| PC-O [36:2] | 0.629 ± 0.033 | 0.610 ± 0.041 | 0.631 ± 0.048 | 0.641 ± 0.029 |
| PC-O [36:1] | 0.329 ± 0.034 | 0.331 ± 0.014 | 0.335 ± 0.020 | 0.359 ± 0.031 |
| PC-O [38:6] | 0.794 ± 0.059 | 0.812 ± 0.054 | 0.846 ± 0.090 | 0.834 ± 0.046 |
| PC-O [38:5] | 1.134 ± 0.060 | 1.120 ± 0.088 | 1.151 ± 0.138 | 1.134 ± 0.088 |
| PC-O [38:4] | 0.478 ± 0.050 | 0.439 ± 0.038 | 0.459 ± 0.066 | 0.449 ± 0.032 |
| **PC-O** | 10.620 ± 0.427 | 10.427 ± 0.621 | 10.802 ± 0.945 | 10.996 ± 0.564 |
| LPE [18:1] | 0.012 ± 0.003 | 0.010 ± 0.003 | 0.008 ± 0.001 | 0.006 ± 0.002 * |
| LPE [18:0] | 0.020 ± 0.011 | 0.011 ± 0.003 | 0.007 ± 0.002 | 0.004 ± 0.002 * |
| LPE [22:6] | 0.002 ± 0.002 | 0.008 ± 0.002 ** | 0.005 ± 0.002 | 0.005 ± 0.001 * |
| **LPE** | 0.034 ± 0.015 | 0.029 ± 0.007 | 0.020 ± 0.002 | 0.015 ± 0.002 * |
| PE [32:1] | 0.057 ± 0.012 | 0.060 ± 0.019 | 0.061 ± 0.009 | 0.074 ± 0.010 |
| PE     | PE-PI | PE-Pl | PE-PI | PE-Pl |
|--------|-------|-------|-------|-------|
| PE [34:2] | 0.050 ± 0.016 | 0.102 ± 0.015 ** | 0.084 ± 0.028 | 0.101 ± 0.018 ** |
| PE [34:1] | 0.714 ± 0.059 | 0.768 ± 0.041 | 0.747 ± 0.069 | 0.812 ± 0.037 * |
| PE [36:4] | 0.137 ± 0.007 | 0.194 ± 0.023 ** | 0.184 ± 0.030 * | 0.179 ± 0.039 |
| PE [36:3] | 0.079 ± 0.004 | 0.092 ± 0.005 ** | 0.095 ± 0.019 | 0.097 ± 0.020 |
| PE [36:2] | 0.743 ± 0.074 | 0.797 ± 0.050 | 0.792 ± 0.098 | 0.853 ± 0.058 |
| PE [36:1] | 0.728 ± 0.083 | 0.807 ± 0.042 | 0.773 ± 0.081 | 0.841 ± 0.016 * |
| PE [38:6] | 0.236 ± 0.026 | 0.252 ± 0.025 | 0.251 ± 0.034 | 0.273 ± 0.014 * |
| PE [38:5] | 0.464 ± 0.042 | 0.489 ± 0.036 | 0.485 ± 0.059 | 0.506 ± 0.033 |
| PE [38:4] | 0.659 ± 0.027 | 0.705 ± 0.024 * | 0.702 ± 0.058 | 0.730 ± 0.029 * |
| PE [38:3] | 0.085 ± 0.011 | 0.092 ± 0.017 | 0.112 ± 0.023 | 0.118 ± 0.010 ** |
| PE [38:2] | 0.058 ± 0.007 | 0.072 ± 0.009 * | 0.052 ± 0.026 | 0.074 ± 0.011 |
| PE [40:7] | 0.149 ± 0.005 | 0.204 ± 0.022 ** | 0.212 ± 0.025 ** | 0.231 ± 0.026 ** |
| PE [40:6] | 0.406 ± 0.059 | 0.473 ± 0.031 | 0.476 ± 0.049 | 0.510 ± 0.025 * |
| PE [40:5] | 0.177 ± 0.011 | 0.211 ± 0.017 * | 0.183 ± 0.014 | 0.220 ± 0.023 * |
| PE     | 4.740 ± 0.398 | 5.319 ± 0.280 | 5.210 ± 0.572 | 5.619 ± 0.239 ** |

| PE-PI [34:1] | 0.316 ± 0.038 | 0.349 ± 0.048 | 0.343 ± 0.069 | 0.369 ± 0.033 |
| PE-PI [36:5] | 0.084 ± 0.022 | 0.069 ± 0.007 | 0.073 ± 0.032 | 0.086 ± 0.025 |
| PE-PI [36:4] | 0.467 ± 0.062 | 0.524 ± 0.085 | 0.512 ± 0.114 | 0.538 ± 0.046 |
| PE-PI [36:3] | 0.031 ± 0.013 | 0.051 ± 0.005 * | 0.048 ± 0.011 | 0.057 ± 0.004 ** |
| PE-PI [36:2] | 0.142 ± 0.022 | 0.148 ± 0.021 | 0.152 ± 0.050 | 0.159 ± 0.015 |
| PE-PI [36:1] | 0.150 ± 0.016 | 0.166 ± 0.040 | 0.176 ± 0.064 | 0.197 ± 0.028 * |
| PE-PI [38:6] | 0.599 ± 0.066 | 0.692 ± 0.083 | 0.697 ± 0.128 | 0.742 ± 0.057 * |
| PE-PI [38:5] | 0.328 ± 0.014 | 0.455 ± 0.063 ** | 0.426 ± 0.102 | 0.470 ± 0.036 ** |
| PE-PI [38:4] | 0.339 ± 0.066 | 0.371 ± 0.079 | 0.354 ± 0.087 | 0.364 ± 0.027 |
| PE-PI [40:7] | 0.099 ± 0.048 | 0.094 ± 0.028 | 0.093 ± 0.094 | 0.101 ± 0.055 |
| PE-PI [40:6] | 0.173 ± 0.025 | 0.254 ± 0.049 * | 0.227 ± 0.132 | 0.264 ± 0.055 * |
| PE-PI [40:5] | 0.147 ± 0.033 | 0.203 ± 0.037 | 0.208 ± 0.034 * | 0.208 ± 0.017 * |
| PE-PI [40:4] | 0.087 ± 0.010 | 0.109 ± 0.013 * | 0.111 ± 0.006 ** | 0.104 ± 0.022 |

** PE-PI 2.962 ± 0.248 | 3.485 ± 0.484 | 3.419 ± 0.828 | 3.658 ± 0.212 **
|      |          |          |          |          |
|------|----------|----------|----------|----------|
| LPI [16:0] | 0.017 ± 0.005 | 0.018 ± 0.007 | 0.020 ± 0.002 | 0.018 ± 0.003 |
| LPI [18:1] | 0.036 ± 0.009 | 0.040 ± 0.006 | 0.040 ± 0.003 | 0.040 ± 0.005 |
| LPI [18:0] | 0.078 ± 0.007 | 0.090 ± 0.014 | 0.086 ± 0.009 | 0.086 ± 0.015 |
| LPI | 0.131 ± 0.022 | 0.147 ± 0.021 | 0.146 ± 0.014 | 0.145 ± 0.023 |
| PI [32:1] | 0.049 ± 0.008 | 0.051 ± 0.005 | 0.054 ± 0.011 | 0.059 ± 0.009 |
| PI [34:2] | 0.095 ± 0.010 | 0.108 ± 0.012 | 0.113 ± 0.023 | 0.123 ± 0.009 ** |
| PI [34:1] | 0.654 ± 0.118 | 0.740 ± 0.092 | 0.723 ± 0.113 | 0.781 ± 0.061 |
| PI [36:5] | 0.056 ± 0.027 | 0.078 ± 0.023 | 0.077 ± 0.030 | 0.084 ± 0.024 |
| PI [36:4] | 0.417 ± 0.020 | 0.447 ± 0.032 | 0.448 ± 0.039 | 0.449 ± 0.026 |
| PI [36:3] | 0.116 ± 0.008 | 0.128 ± 0.015 | 0.127 ± 0.020 | 0.132 ± 0.012 |
| PI [36:2] | 0.582 ± 0.058 | 0.655 ± 0.033 | 0.624 ± 0.069 | 0.673 ± 0.066 |
| PI [36:1] | 0.264 ± 0.011 | 0.305 ± 0.013 ** | 0.311 ± 0.033 * | 0.345 ± 0.035 ** |
| PI [38:6] | 0.079 ± 0.013 | 0.088 ± 0.014 | 0.088 ± 0.014 | 0.087 ± 0.011 |
| PI [38:5] | 0.365 ± 0.020 | 0.399 ± 0.032 | 0.394 ± 0.037 | 0.402 ± 0.036 |
| PI [38:4] | 4.928 ± 0.171 | 5.014 ± 0.374 | 5.057 ± 0.356 | 4.862 ± 0.289 |
| PI [38:3] | 0.269 ± 0.020 | 0.314 ± 0.035 | 0.305 ± 0.043 | 0.318 ± 0.037 |
| PI [38:2] | 0.017 ± 0.002 | 0.021 ± 0.001 * | 0.017 ± 0.003 | 0.024 ± 0.007 |
| PI [40:6] | 0.167 ± 0.014 | 0.181 ± 0.018 | 0.180 ± 0.020 | 0.185 ± 0.010 |
| PI [40:5] | 0.211 ± 0.010 | 0.227 ± 0.019 | 0.230 ± 0.017 | 0.227 ± 0.012 |
| PI [40:4] | 0.118 ± 0.008 | 0.129 ± 0.015 | 0.125 ± 0.011 | 0.125 ± 0.006 |
| PI | 8.389 ± 0.344 | 8.886 ± 0.553 | 8.873 ± 0.805 | 8.877 ± 0.387 |
| PS [32:1] | 0.023 ± 0.004 | 0.023 ± 0.002 | 0.022 ± 0.002 | 0.023 ± 0.002 |
| PS [34:2] | 0.024 ± 0.003 | 0.019 ± 0.004 | 0.020 ± 0.003 | 0.020 ± 0.002 |
| PS [34:1] | 0.525 ± 0.042 | 0.552 ± 0.038 | 0.553 ± 0.072 | 0.574 ± 0.044 |
| PS [36:3] | 0.025 ± 0.003 | 0.023 ± 0.002 | 0.024 ± 0.003 | 0.025 ± 0.003 |
| PS [36:2] | 0.267 ± 0.020 | 0.302 ± 0.030 | 0.304 ± 0.037 | 0.326 ± 0.034 * |
| PS [36:1] | 2.027 ± 0.134 | 2.169 ± 0.071 | 2.215 ± 0.152 | 2.269 ± 0.172 |
|        |        |        |        |        |
|--------|--------|--------|--------|--------|
| PS 38:6 | 0.022 ± 0.003 | 0.018 ± 0.003 | 0.015 ± 0.002 ** | 0.013 ± 0.002 ** |
| PS 38:5 | 0.020 ± 0.003 | 0.031 ± 0.006 * | 0.026 ± 0.004 * | 0.030 ± 0.005 * |
| PS 38:3 | 0.127 ± 0.018 | 0.161 ± 0.037 | 0.149 ± 0.022 | 0.176 ± 0.022 * |
| PS 38:2 | 0.025 ± 0.004 | 0.034 ± 0.005 * | 0.043 ± 0.006 ** | 0.038 ± 0.006 * |
| PS 38:1 | 0.043 ± 0.003 | 0.047 ± 0.020 | 0.064 ± 0.009 ** | 0.054 ± 0.012 |
| PS 38:0 | 0.038 ± 0.019 | 0.066 ± 0.051 | 0.113 ± 0.036 * | 0.072 ± 0.033 |
| PS 40:6 | 0.283 ± 0.020 | 0.324 ± 0.027 | 0.325 ± 0.025 * | 0.344 ± 0.036 * |
| PS 40:5 | 0.027 ± 0.027 | 0.098 ± 0.029 * | 0.092 ± 0.029 * | 0.096 ± 0.036 * |
| PS 40:4 | 0.104 ± 0.018 | 0.125 ± 0.019 | 0.108 ± 0.028 | 0.118 ± 0.014 |
| PS 40:2 | 0.020 ± 0.005 | 0.023 ± 0.009 | 0.029 ± 0.011 | 0.031 ± 0.008 |
| PS 40:1 | 0.051 ± 0.004 | 0.068 ± 0.015 | 0.072 ± 0.010 ** | 0.079 ± 0.015 ** |
| PS 42:2 | 0.031 ± 0.002 | 0.039 ± 0.003 ** | 0.038 ± 0.001 ** | 0.041 ± 0.005 ** |
| PS 42:1 | 0.042 ± 0.008 | 0.051 ± 0.019 | 0.056 ± 0.014 | 0.066 ± 0.020 |
| PS     | 3.724 ± 0.194 | 4.172 ± 0.177 * | 4.267 ± 0.297 * | 4.394 ± 0.229 ** |
|        |        |        |        |        |
|        |        |        |        |        |
|        |        |        |        |        |
| PG 32:1 | 0.001 ± 0.000 | 0.003 ± 0.001 ** | 0.003 ± 0.001 * | 0.003 ± 0.001 |
| PG 34:2 | 0.010 ± 0.001 | 0.012 ± 0.004 | 0.014 ± 0.002 * | 0.013 ± 0.003 |
| PG 34:1 | 0.308 ± 0.027 | 0.303 ± 0.025 | 0.296 ± 0.022 | 0.303 ± 0.013 |
| PG 36:3 | 0.032 ± 0.002 | 0.034 ± 0.004 | 0.033 ± 0.002 | 0.038 ± 0.004 * |
| PG 36:2 | 0.190 ± 0.011 | 0.223 ± 0.008 ** | 0.218 ± 0.024 | 0.241 ± 0.020 ** |
| PG 36:1 | 0.153 ± 0.007 | 0.152 ± 0.012 | 0.162 ± 0.032 | 0.150 ± 0.011 |
| PG 38:6 | 0.005 ± 0.003 | 0.006 ± 0.002 | 0.007 ± 0.002 | 0.005 ± 0.002 |
| PG 38:3 | 0.006 ± 0.002 | 0.005 ± 0.001 | 0.007 ± 0.002 | 0.006 ± 0.002 |
| PG 40:7 | 0.093 ± 0.009 | 0.113 ± 0.007 * | 0.113 ± 0.011 * | 0.111 ± 0.007 * |
| PG 40:6 | 0.011 ± 0.003 | 0.014 ± 0.004 | 0.014 ± 0.005 | 0.012 ± 0.003 |
| PG 44:12 | 0.008 ± 0.003 | 0.010 ± 0.004 | 0.010 ± 0.004 | 0.007 ± 0.002 |
| PG     | 0.819 ± 0.028 | 0.875 ± 0.029 * | 0.876 ± 0.024 * | 0.889 ± 0.030 * |
|        |        |        |        |        |
|        |        |        |        |        |
|        |        |        |        |        |
| PA 32:1 | 0.001 ± 0.001 | 0.001 ± 0.000 | 0.004 ± 0.001 ** | 0.002 ± 0.001 |
| PA 34:2 | 0.003 ± 0.002 | 0.004 ± 0.001 | 0.005 ± 0.001 | 0.005 ± 0.003 |
|                | Value 1     | Value 2     | Value 3     | Value 4     |
|----------------|------------|------------|------------|------------|
| PA [34:1]     | 0.077 ± 0.004 | 0.068 ± 0.008 | 0.102 ± 0.005 ** | 0.115 ± 0.018 ** |
| PA [36:2]     | 0.006 ± 0.004 | 0.010 ± 0.007 | 0.021 ± 0.006 ** | 0.029 ± 0.009 ** |
| PA [36:1]     | 0.004 ± 0.001 | 0.005 ± 0.001 | 0.007 ± 0.002 *  | 0.007 ± 0.002   |
| PA            | 0.091 ± 0.010 | 0.089 ± 0.013 | 0.138 ± 0.009 ** | 0.157 ± 0.023 ** |
| CL [68:5]     | 0.013 ± 0.008 | 0.008 ± 0.000 | 0.020 ± 0.006   | 0.005 ± 0.001   |
| CL [68:4]     | 0.037 ± 0.009 | 0.045 ± 0.011 | 0.033 ± 0.005   | 0.054 ± 0.009 * |
| CL [68:3]     | 0.026 ± 0.007 | 0.036 ± 0.010 | 0.035 ± 0.010   | 0.054 ± 0.007 **|
| CL [68:2]     | 0.042 ± 0.017 | 0.076 ± 0.030 | 0.073 ± 0.008 * | 0.082 ± 0.029   |
| CL [70:6]     | 0.053 ± 0.013 | 0.079 ± 0.009 * | 0.079 ± 0.008   | 0.085 ± 0.005 **|
| CL [70:5]     | 0.114 ± 0.028 | 0.180 ± 0.010 ** | 0.172 ± 0.028 * | 0.200 ± 0.006 **|
| CL [70:4]     | 0.091 ± 0.008 | 0.087 ± 0.016 | 0.093 ± 0.030   | 0.116 ± 0.008 **|
| CL [70:3]     | 0.027 ± 0.008 | 0.043 ± 0.007 * | 0.045 ± 0.011 * | 0.045 ± 0.005 **|
| CL [70:2]     | 0.015 ± 0.003 | 0.032 ± 0.015 | 0.036 ± 0.009 ** | 0.035 ± 0.009 **|
| CL [72:8]     | 0.008 ± 0.002 | 0.012 ± 0.004 | 0.011 ± 0.004   | 0.020 ± 0.001 **|
| CL [72:7]     | 0.026 ± 0.010 | 0.024 ± 0.008 | 0.024 ± 0.015   | 0.051 ± 0.002 **|
| CL [72:6]     | 0.095 ± 0.010 | 0.123 ± 0.007 ** | 0.123 ± 0.011 * | 0.121 ± 0.042   |
| CL [72:5]     | 0.047 ± 0.013 | 0.076 ± 0.005 ** | 0.068 ± 0.006 * | 0.076 ± 0.003 **|
| CL [72:4]     | 0.028 ± 0.008 | 0.043 ± 0.006 * | 0.049 ± 0.008 ** | 0.048 ± 0.002 **|
| CL [74:8]     | 0.017 ± 0.006 | 0.023 ± 0.014 | 0.016 ± 0.008   | 0.017 ± 0.009   |
| CL [74:7]     | 0.042 ± 0.018 | 0.060 ± 0.006 | 0.064 ± 0.023   | 0.078 ± 0.005 **|
| CL [74:6]     | 0.028 ± 0.016 | 0.035 ± 0.009 | 0.042 ± 0.022   | 0.026 ± 0.007   |
| CL            | 0.710 ± 0.129 | 0.982 ± 0.051 ** | 0.983 ± 0.111 * | 1.112 ± 0.023 **|
| SM [34:2:2]   | 0.432 ± 0.067 | 0.454 ± 0.039 | 0.451 ± 0.022   | 0.471 ± 0.033   |
| SM [34:1:2]   | 7.114 ± 0.484 | 7.083 ± 0.272 | 7.173 ± 0.230   | 7.390 ± 0.429   |
| SM [40:1:2]   | 0.135 ± 0.022 | 0.145 ± 0.005 | 0.141 ± 0.017   | 0.154 ± 0.014   |
| SM [42:2:2]   | 0.701 ± 0.128 | 0.710 ± 0.055 | 0.745 ± 0.043   | 0.769 ± 0.078   |
| SM [42:1:2]   | 0.302 ± 0.022 | 0.277 ± 0.016 | 0.275 ± 0.005   | 0.277 ± 0.017   |
| SM            | 8.685 ± 0.678 | 8.668 ± 0.369 | 8.786 ± 0.289   | 9.061 ± 0.551   |
|           | Cer [34:1:2] | Cer [40:2:2] | Cer [40:1:2] | Cer [41:1:2] | Cer [42:2:2] | Cer [42:1:2] | Cer  |
|-----------|-------------|-------------|-------------|-------------|-------------|-------------|------|
|           | 0.088 ± 0.002 | 0.001 ± 0.001 | 0.009 ± 0.002 | 0.003 ± 0.001 | 0.067 ± 0.002 | 0.057 ± 0.005 | 0.226 ± 0.007 |
|           | 0.112 ± 0.003 ** | 0.002 ± 0.000 | 0.013 ± 0.001 * | 0.004 ± 0.002 | 0.086 ± 0.004 ** | 0.065 ± 0.006 | 0.283 ± 0.014 ** |
|           | 0.106 ± 0.006 ** | 0.002 ± 0.001 | 0.014 ± 0.002 * | 0.004 ± 0.001 | 0.083 ± 0.007 ** | 0.067 ± 0.007 | 0.276 ± 0.019 ** |
|           | 0.113 ± 0.005 ** | 0.002 ± 0.000 | 0.017 ± 0.002 ** | 0.004 ± 0.001 | 0.094 ± 0.007 ** | 0.068 ± 0.008 | 0.298 ± 0.018 ** |

|           | GlCer [34:1:2] | GlCer [40:1:2] | GlCer [41:1:2] | GlCer [42:2:2] | GlCer [42:1:2] | GlCer  |
|-----------|--------------|--------------|--------------|--------------|--------------|-------|
|           | 0.144 ± 0.015 | 0.009 ± 0.001 | 0.001 ± 0.001 | 0.032 ± 0.004 | 0.026 ± 0.007 | 0.211 ± 0.019 |
|           | 0.141 ± 0.002 | 0.012 ± 0.002 * | 0.002 ± 0.001 | 0.039 ± 0.002 * | 0.028 ± 0.003 | 0.222 ± 0.006 |
|           | 0.155 ± 0.008 | 0.009 ± 0.001 | 0.003 ± 0.002 | 0.039 ± 0.005 * | 0.030 ± 0.002 | 0.235 ± 0.009 |
|           | 0.162 ± 0.007 | 0.009 ± 0.003 | 0.001 ± 0.000 * | 0.043 ± 0.005 * | 0.034 ± 0.004 | 0.249 ± 0.013 * |

|           | GM3 [34:1:2] | GM3 [40:1:2] | GM3 [41:2:2] | GM3 [42:2:2] | GM3 [42:1:2] | GM3  |
|-----------|-------------|-------------|-------------|-------------|-------------|------|
|           | 1.793 ± 0.085 | 0.189 ± 0.019 | 0.014 ± 0.003 | 0.841 ± 0.041 | 0.473 ± 0.018 | 3.310 ± 0.152 |
|           | 1.749 ± 0.095 | 0.172 ± 0.037 | 0.010 ± 0.009 | 0.900 ± 0.009 * | 0.489 ± 0.011 | 3.319 ± 0.124 |
|           | 1.807 ± 0.091 | 0.203 ± 0.021 | 0.019 ± 0.006 | 0.894 ± 0.012 * | 0.510 ± 0.021 * | 3.434 ± 0.093 |
|           | 1.771 ± 0.125 | 0.182 ± 0.036 | 0.015 ± 0.001 | 0.908 ± 0.005 * | 0.507 ± 0.010 * | 3.383 ± 0.162 |

|           | GM3          |
|-----------|-------------|
|           | 3.310 ± 0.152 | 3.319 ± 0.124 | 3.434 ± 0.093 | 3.383 ± 0.162 |

|           | Class        |
|-----------|-------------|
|           | LPC         | PC           | PC-O         | LPE         | PE          | PE-Pl       |
|           | 0.606 ± 0.059 | 54.741 ± 2.105 | 10.620 ± 0.427 | 0.034 ± 0.015 | 4.740 ± 0.398 | 2.962 ± 0.248 |
|           | 0.439 ± 0.062 ** | 52.659 ± 0.916 | 10.427 ± 0.621 | 0.029 ± 0.007 | 5.319 ± 0.280 | 3.485 ± 0.484 |
|           | 0.273 ± 0.035 ** | 52.261 ± 1.764 | 10.802 ± 0.945 | 0.020 ± 0.002 | 5.210 ± 0.572 | 3.419 ± 0.828 |
|           | 0.222 ± 0.030 ** | 50.925 ± 1.162 * | 10.996 ± 0.564 | 0.015 ± 0.002 * | 5.619 ± 0.239 ** | 3.658 ± 0.212 ** |
|   | 0.131 ± 0.022 | 0.147 ± 0.021 | 0.146 ± 0.014 | 0.145 ± 0.023 |
|---|---|---|---|---|
| PI | 8.389 ± 0.344 | 8.866 ± 0.553 | 8.873 ± 0.805 | 8.877 ± 0.387 |
| PS | 3.724 ± 0.194 | 4.172 ± 0.177 * | 4.267 ± 0.297 * | 4.394 ± 0.229 ** |
| PG | 0.819 ± 0.028 | 0.875 ± 0.029 * | 0.876 ± 0.024 * | 0.889 ± 0.030 * |
| PA | 0.091 ± 0.010 | 0.089 ± 0.013 | 0.138 ± 0.009 ** | 0.157 ± 0.023 ** |
| CL | 0.710 ± 0.129 | 0.982 ± 0.051 ** | 0.983 ± 0.111 * | 1.112 ± 0.023 ** |
| SM | 8.685 ± 0.678 | 8.668 ± 0.369 | 8.786 ± 0.289 | 9.061 ± 0.551 |
| Cer | 0.226 ± 0.007 | 0.283 ± 0.014 ** | 0.276 ± 0.019 ** | 0.298 ± 0.018 ** |
| GICer | 0.211 ± 0.019 | 0.222 ± 0.006 | 0.235 ± 0.009 | 0.249 ± 0.013 * |
| GM3 | 3.310 ± 0.152 | 3.319 ± 0.124 | 3.434 ± 0.093 | 3.383 ± 0.162 |