

Supplementary Material

Gemfibrozil-induced intracellular Triglyceride increase in SH-SY5Y, HEK and Calu-3 cells

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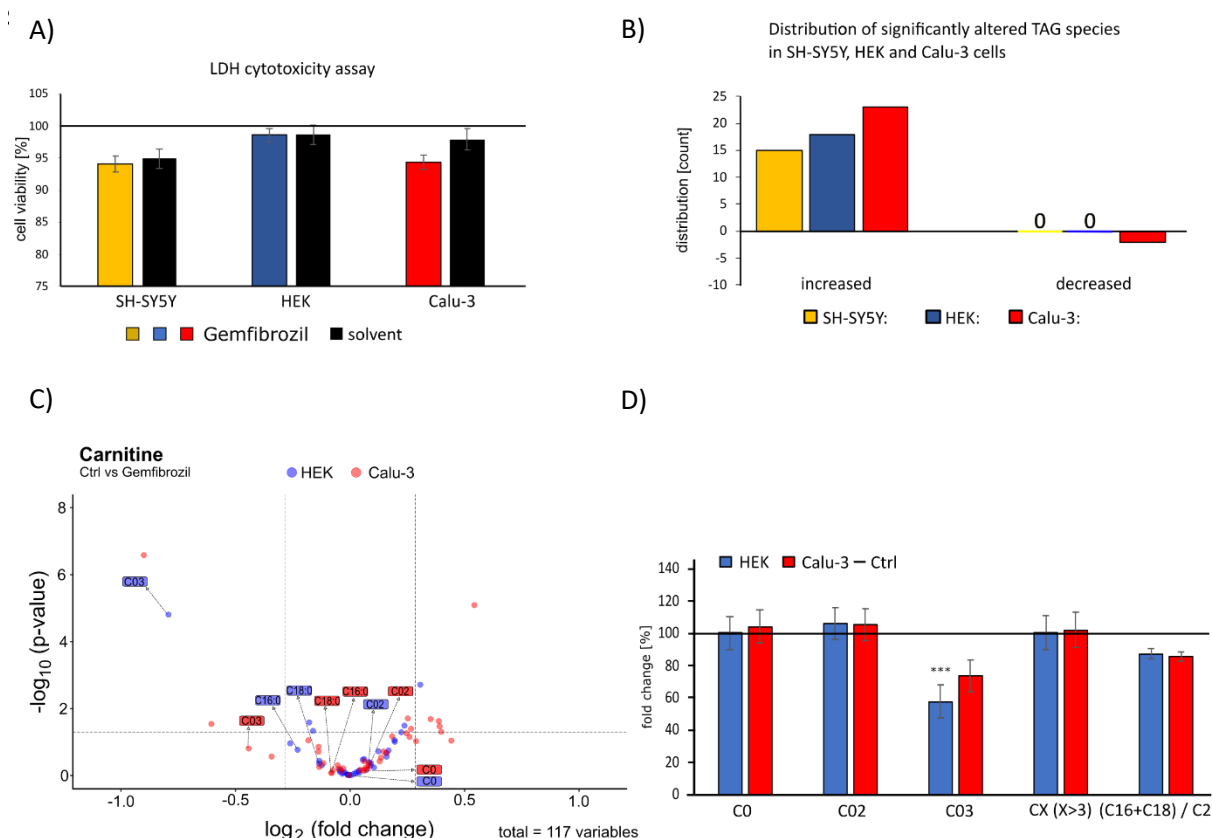
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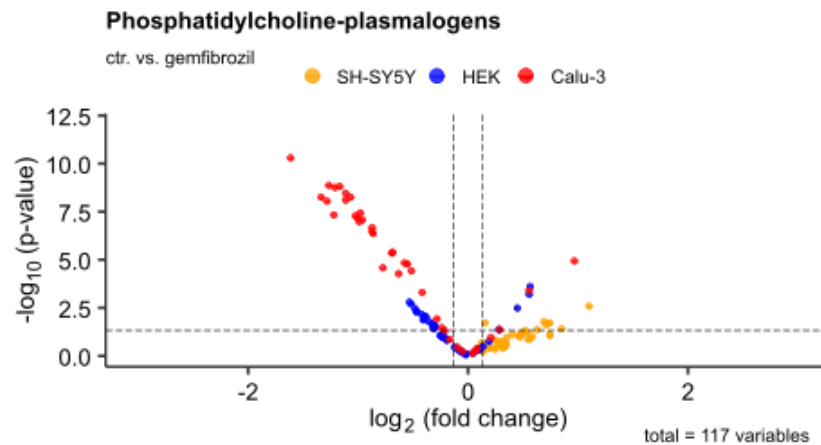
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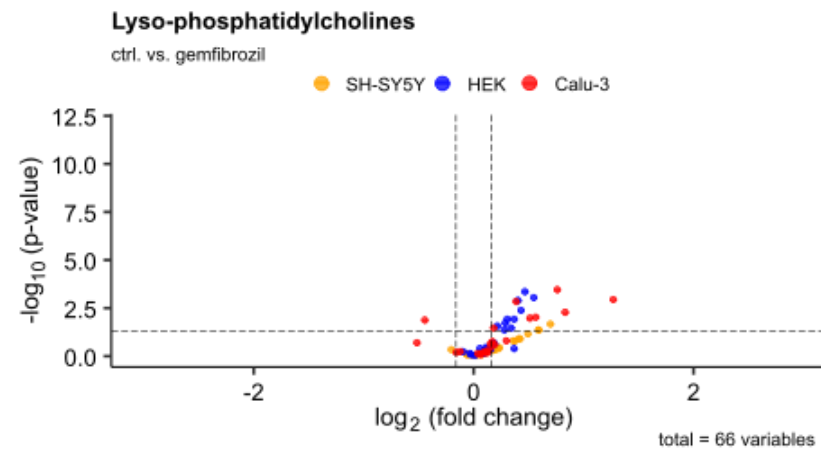


Supplemental figure S1: A) Investigation of cell viability after incubation of SH-SY5Y, HEK and Calu-3 cells with 100 $\mu\text{mol/l}$ gemfibrozil for 72 h (0 + 24 + 48 h) via cytotoxicity detection assay. Care was taken not to exceed 6% of cytotoxicity. **B)** A bar chart shows the distribution of significantly altered TAG-species after gemfibrozil treatment in SH-SY5Y, HEK and Calu-3 cells. All species were considered that fell below the significance level of $p = 0.05$. On the left side all significantly increased species for each cell type are shown. On the right side are all significantly decreased species illustrated. **C)** Changed carnitine levels in HEK and Calu-3 cells after gemfibrozil treatment compared to cells treated with the solvent control (ethanol) are illustrated in the volcano plot. The fold change (x-axis) of each of the 41 examined carnitine species (dots) was plotted against the corresponding logarithmic p-value (y-axis). A detailed structure of the volcano plot is explained in the legend of figure 1. **D)** The bar chart shows the changes in C0, C2, C3, CX ($X > 3$) and (C16 + C18)/C2 ratio in gemfibrozil treated HEK and Calu-3 cells compared to cells treated with the solvent control (ethanol), which is represented as the horizontal line and set as 100%. The obtained data were calculated by using the students t-test comparing gemfibrozil-treated cells against the control of each cell line.

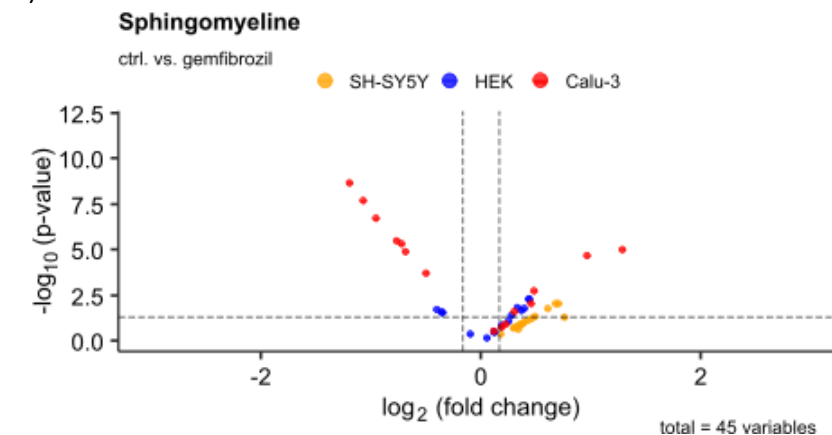
A)



B)



C)



Supplemental figure S2: A) - C) changed Phosphatidylcholine-plasmalogen (PCae), Lyso-phosphatidylcholine (Lyso-PC) and Sphingomyelin (SM) levels in SH-SY5Y, HEK and Calu-3 cells after gemfibrozil treatment compared to cells treated with the solvent control (ethanol) are illustrated in the volcano plots (A – C), where the fold change (x-axis) of each of the examined lipid species (dots) was plotted against the corresponding logarithmic p-value (y-axis). All three cell lines are shown in one volcano plot with three different colors: SH-SY5Y in orange, HEK in blue and Calu-3 in red.

4.1. Chemicals, Reagents, Standards

HPLC-grade pyridine, phenyl isothiocyanate (PITC) and ammonium acetate used for lipid extraction were purchased from Merck (Darmstadt, Germany). All other chemicals including high performance liquid chromatography (HPLC)-grade water, ethanol, and methanol were purchased from Fisher Scientific (Schwerte, Germany), if not stated otherwise. Gemfibrozil was kindly provided in form of Gevilon® 600 mg (from Pfizer) by the Kronen-Apotheke, 09212 Limbach-Oberfrohna, Germany. For normalization the following standards from Avanti Polar Lipids were used: 06:0 PC (DHPC), 19:0 Lyso PC, 06:0 SM (d18:1/6:0), and Splash II Lipidomix Mass Spec Internal Standard. The carnitine standards octanoyl-L-carnitine d3 and palmitoyl-L-carnitine d3 were purchased from Supelco Analytical.

4.6. Targeted Shotgun Mass Spectrometry

Table S5 lists all validation parameters required for shotgun mass spectrometry. A detailed description of the validation and their respective origin data can be found in Lauer et al. [32].

Table S5: Overview of the used validation parameters for Mass spectrometry analysis in this study. For the data obtained in this work, the experimental conditions of Lauer et. al. [32] were adopted. The focus was on average extraction efficiency, linearity for each lipid class, intra- and inter-day variance, and potential matrix effects.

Validation parameter	result
Average extraction efficiency	> 80.7 %
Intra-day variance (extraction)	3.9 %
Linearity of PC aa	$R^2 = 0.98$
Linearity of PC ae	$R^2 = 0.97$
Linearity of Lyso-PC	$R^2 = 0.96$
Linearity of SM	$R^2 = 0.98$
Linearity of C16	$R^2 = 0.99$
Linearity of TAG	$R^2 = 0.99$
Intra- and inter-day variance of lipid detection	6.5 %
Potential matrix effects	< 3.2 %

Group analysis

Table S6: Results of group analysis of the raw data of **triglycerides** (TAG) total. In this analysis we calculated via a Two-Factor ANOVA (Factor 1 treatment (control vs gemfibrozil) and Factor 2 cell line (Calu-3, HEK and SH-SY5Y). Following this we calculated the p-values of each combination via the post hoc test TukeyHSD (calculated p-values shown in the table below). Prior to this we used the LeveneTest to determine if the homogeneity of variances was given for this model (see p-value LeveneTest: $p = 0.5558$).

	SH-SY5Y ctrl	SH-SY5Y gemfi	HEK ctrl	HEK gemfi	Calu-3 ctrl	Calu-3 gemfi
SH-SY5Y ctrl	-	$p = 6.06 \times 10^{-4}$	$p = 8.57 \times 10^{-1}$	$p = 9.72 \times 10^{-11}$	$p = 8.09 \times 10^{-11}$	$p = 8.16 \times 10^{-13}$
SH-SY5Y gemfi	$p = 6.06 \times 10^{-4}$	-	$p = 1.23 \times 10^{-2}$	$p = 1.10 \times 10^{-4}$	$p = 9.00 \times 10^{-5}$	$p = 8.16 \times 10^{-13}$
HEK ctrl	$p = 8.57 \times 10^{-1}$	$p = 1.23 \times 10^{-2}$	-	$p = 1.31 \times 10^{-9}$	$p = 1.08 \times 10^{-9}$	$p = 8.15 \times 10^{-13}$
HEK gemfi	$p = 9.72 \times 10^{-11}$	$p = 1.10 \times 10^{-4}$	$p = 1.31 \times 10^{-9}$	-	$p = 9.99 \times 10^{-1}$	$p = 8.33 \times 10^{-13}$
Calu-3 ctrl	$p = 8.09 \times 10^{-11}$	$p = 9.00 \times 10^{-5}$	$p = 1.08 \times 10^{-9}$	$p = 9.99 \times 10^{-1}$	-	$p = 8.34 \times 10^{-13}$
Calu-3 gemfi	$p = 8.16 \times 10^{-13}$	$p = 8.16 \times 10^{-13}$	$p = 8.15 \times 10^{-13}$	$p = 8.33 \times 10^{-13}$	$p = 8.34 \times 10^{-13}$	-

Table S7: Results of group analysis of the raw data of **acylcarnitines** (C) total. In this analysis we calculated via a Two-Factor ANOVA (Factor 1 treatment (control vs gemfibrozil) and Factor 2 cell line (Calu-3, HEK and SH-SY5Y). Following this we calculated the p-values of each combination via the post hoc test TukeyHSD (calculated p-values shown in the table below). Prior to this we used the LeveneTest to determine if the homogeneity of variances was given for this model (see p-value LeveneTest: $p = 0.2301$).

	SH-SY5Y ctrl	SH-SY5Y gemfi	HEK ctrl	HEK gemfi	Calu-3 ctrl	Calu-3 gemfi
SH-SY5Y ctrl	-	$p = 9.99 \times 10^{-1}$	$p = 8.90 \times 10^{-1}$	$p = 9.10 \times 10^{-1}$	$p = 6.54 \times 10^{-3}$	$p = 2.30 \times 10^{-3}$
SH-SY5Y gemfi	$p = 9.99 \times 10^{-1}$	-	$p = 9.21 \times 10^{-1}$	$p = 9.39 \times 10^{-1}$	$p = 3.08 \times 10^{-3}$	$p = 1.01 \times 10^{-3}$
HEK ctrl	$p = 8.90 \times 10^{-1}$	$p = 9.21 \times 10^{-1}$	-	$p = 9.99 \times 10^{-1}$	$p = 1.53 \times 10^{-4}$	$p = 4.66 \times 10^{-5}$
HEK gemfi	$p = 9.10 \times 10^{-1}$	$p = 9.39 \times 10^{-1}$	$p = 9.99 \times 10^{-1}$	-	$p = 1.85 \times 10^{-4}$	$p = 5.64 \times 10^{-5}$
Calu-3 ctrl	$p = 6.54 \times 10^{-3}$	$p = 3.08 \times 10^{-3}$	$p = 1.53 \times 10^{-4}$	$p = 1.85 \times 10^{-4}$	-	$p = 9.99 \times 10^{-1}$
Calu-3 gemfi	$p = 2.30 \times 10^{-3}$	$p = 1.01 \times 10^{-3}$	$p = 4.66 \times 10^{-5}$	$p = 5.64 \times 10^{-5}$	$p = 9.99 \times 10^{-1}$	-

Table S8: Results of group analysis of the raw data of **phosphatidylcholines** (PC aa) total. In this analysis we calculated via a Two-Factor ANOVA (Factor 1 treatment (control vs gemfibrozil) and Factor 2 cell line (Calu-3, HEK and SH-SY5Y). Following this we calculated the p-values of each combination via the post hoc test TukeyHSD (calculated p-values shown in the table below). Prior to this we used the LeveneTest to determine if the homogeneity of variances was given for this model (see p-value LeveneTest: $p = 0.2834$).

	SH-SY5Y ctrl	SH-SY5Y gemfi	HEK ctrl	HEK gemfi	Calu-3 ctrl	Calu-3 gemfi
SH-SY5Y ctrl	-	$p = 5.35 \times 10^{-1}$	$p = 9.99 \times 10^{-1}$	$p = 5.63 \times 10^{-1}$	$p = 2.86 \times 10^{-1}$	$p = 4.77 \times 10^{-3}$
SH-SY5Y gemfi	$p = 5.35 \times 10^{-1}$	-	$p = 5.86 \times 10^{-1}$	$p = 1.000$	$p = 9.97 \times 10^{-1}$	$p = 2.42 \times 10^{-1}$
HEK ctrl	$p = 9.99 \times 10^{-1}$	$p = 5.86 \times 10^{-1}$	-	$p = 6.15 \times 10^{-1}$	$p = 3.18 \times 10^{-1}$	$p = 4.83 \times 10^{-3}$
HEK gemfi	$p = 5.63 \times 10^{-1}$	$p = 1.000$	$p = 6.15 \times 10^{-1}$	-	$p = 9.96 \times 10^{-1}$	$p = 2.23 \times 10^{-1}$
Calu-3 ctrl	$p = 2.86 \times 10^{-1}$	$p = 9.97 \times 10^{-1}$	$p = 3.18 \times 10^{-1}$	$p = 9.96 \times 10^{-1}$	-	$p = 4.84 \times 10^{-1}$
Calu-3 gemfi	$p = 4.77 \times 10^{-3}$	$p = 2.42 \times 10^{-1}$	$p = 4.83 \times 10^{-3}$	$p = 2.23 \times 10^{-1}$	$p = 4.84 \times 10^{-1}$	-

Table S9: Results of group analysis of the raw data of **phosphatidylcholines-plasmalogens** (PC ae) total. In this analysis we calculated via a Two-Factor ANOVA (Factor 1 treatment (control vs gemfibrozil) and Factor 2 cell line (Calu-3, HEK and SH-SY5Y). Following this we calculated the p-values of each combination via the post hoc test TukeyHSD (calculated p-values shown in the table below). Prior to this we used the LeveneTest to determine if the homogeneity of variances was given for this model. (See p-value LeveneTest: $p = 0.3274$).

	SH-SY5Y ctrl	SH-SY5Y gemfi	HEK ctrl	HEK gemfi	Calu-3 ctrl	Calu-3 gemfi
SH-SY5Y ctrl	-	$p = 3.47 \times 10^{-1}$	$p = 3.18 \times 10^{-8}$	$p = 2.61 \times 10^{-8}$	$p \leq 1.88 \times 10^{-12}$	$p = 7.99 \times 10^{-11}$
SH-SY5Y gemfi	$p = 3.47 \times 10^{-1}$	-	$p = 1.18 \times 10^{-5}$	$p = 9.58 \times 10^{-6}$	$p = 1.47 \times 10^{-10}$	$p = 1.81 \times 10^{-8}$
HEK ctrl	$p = 3.18 \times 10^{-8}$	$p = 1.18 \times 10^{-5}$	-	$p = 9.99 \times 10^{-1}$	$p = 1.16 \times 10^{-2}$	$p = 3.58 \times 10^{-1}$
HEK gemfi	$p = 2.61 \times 10^{-8}$	$p = 9.58 \times 10^{-6}$	$p = 9.99 \times 10^{-1}$	-	$p = 1.38 \times 10^{-2}$	$p = 3.94 \times 10^{-1}$
Calu-3 ctrl	$p \leq 1.88 \times 10^{-12}$	$p = 1.47 \times 10^{-10}$	$p = 1.16 \times 10^{-2}$	$p = 1.38 \times 10^{-2}$	-	$p = 6.33 \times 10^{-1}$
Calu-3 gemfi	$p = 7.99 \times 10^{-11}$	$p = 1.81 \times 10^{-8}$	$p = 3.58 \times 10^{-1}$	$p = 3.94 \times 10^{-1}$	$p = 6.33 \times 10^{-1}$	-

Table S10: Results of group analysis of the raw data of **Lyso-phosphatidylcholines** (Lyso-PC) total. In this analysis we calculated via a Two-Factor ANOVA (Factor 1 treatment (control vs gemfibrozil) and Factor 2 cell line (Calu-3, HEK and SH-SY5Y). Following this we calculated the p-values of each combination via the post hoc test TukeyHSD (calculated p-values shown in the table below). Prior to this we used the LeveneTest to determine if the homogeneity of variances was given for this model (see p-value LeveneTest: $p = 0.1611$).

	SH-SY5Y ctrl	SH-SY5Y gemfi	HEK ctrl	HEK gemfi	Calu-3 ctrl	Calu-3 gemfi
SH-SY5Y ctrl	-	$p = 6.89 \times 10^{-1}$	$p = 1.11 \times 10^{-5}$	$p = 8.29 \times 10^{-10}$	$p = 8.99 \times 10^{-1}$	$p = 6.38 \times 10^{-3}$
SH-SY5Y gemfi	$p = 6.89 \times 10^{-1}$	-	$p = 7.15 \times 10^{-4}$	$p = 3.65 \times 10^{-8}$	$p = 9.99 \times 10^{-1}$	$p = 1.84 \times 10^{-1}$
HEK ctrl	$p = 1.11 \times 10^{-5}$	$p = 7.15 \times 10^{-4}$	-	$p = 4.06 \times 10^{-2}$	$p = 1.88 \times 10^{-4}$	$p = 2.97 \times 10^{-1}$
HEK gemfi	$p = 8.29 \times 10^{-10}$	$p = 3.65 \times 10^{-8}$	$p = 4.06 \times 10^{-2}$	-	$p = 9.33 \times 10^{-9}$	$p = 8.32 \times 10^{-5}$
Calu-3 ctrl	$p = 8.99 \times 10^{-1}$	$p = 9.99 \times 10^{-1}$	$p = 1.88 \times 10^{-4}$	$p = 9.33 \times 10^{-9}$	-	$p = 7.44 \times 10^{-2}$
Calu-3 gemfi	$p = 6.38 \times 10^{-3}$	$p = 1.84 \times 10^{-1}$	$p = 2.97 \times 10^{-1}$	$p = 8.32 \times 10^{-5}$	$p = 7.44 \times 10^{-2}$	-

Table S11: Results of group analysis of the raw data of **Sphingomyelins** (SM) total. In this analysis we calculated via a Two-Factor ANOVA (Factor 1 treatment (control vs gemfibrozil) and Factor 2 cell line (Calu-3, HEK and SH-SY5Y). Following this we calculated the p-values of each combination via the post hoc test TukeyHSD (calculated p-values shown in the table below). Prior to this we used the LeveneTest to determine if the homogeneity of variances was given for this model. (See p-value LeveneTest: $p = 0.1624$).

	SH-SY5Y ctrl	SH-SY5Y gemfi	HEK ctrl	HEK gemfi	Calu-3 ctrl	Calu-3 gemfi
SH-SY5Y ctrl	-	$p = 1.47 \times 10^{-1}$	$p = 6.61 \times 10^{-2}$	$p = 5.26 \times 10^{-4}$	$p = 1.10 \times 10^{-3}$	$p = 1.24 \times 10^{-4}$
SH-SY5Y gemfi	$p = 1.47 \times 10^{-1}$	-	$p = 9.98 \times 10^{-1}$	$p = 2.68 \times 10^{-1}$	$p = 3.96 \times 10^{-1}$	$p = 1.06 \times 10^{-1}$
HEK ctrl	$p = 6.61 \times 10^{-2}$	$p = 9.98 \times 10^{-1}$	-	$p = 4.99 \times 10^{-1}$	$p = 6.56 \times 10^{-1}$	$p = 2.42 \times 10^{-1}$
HEK gemfi	$p = 5.26 \times 10^{-4}$	$p = 2.68 \times 10^{-1}$	$p = 4.99 \times 10^{-1}$	-	$p = 9.99 \times 10^{-1}$	$p = 9.97 \times 10^{-1}$
Calu-3 ctrl	$p = 1.10 \times 10^{-3}$	$p = 3.96 \times 10^{-1}$	$p = 6.56 \times 10^{-1}$	$p = 9.99 \times 10^{-1}$	-	$p = 9.78 \times 10^{-1}$
Calu-3 gemfi	$p = 1.24 \times 10^{-4}$	$p = 1.06 \times 10^{-1}$	$p = 2.42 \times 10^{-1}$	$p = 9.97 \times 10^{-1}$	$p = 9.78 \times 10^{-1}$	-