Supplemental Material

Sterol biosynthesis inhibition in pregnant women taking prescription medications

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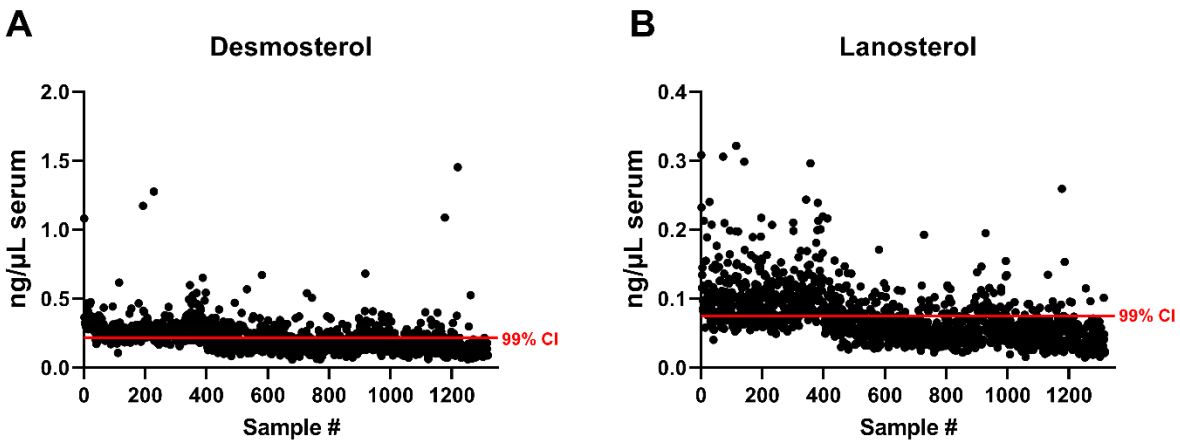
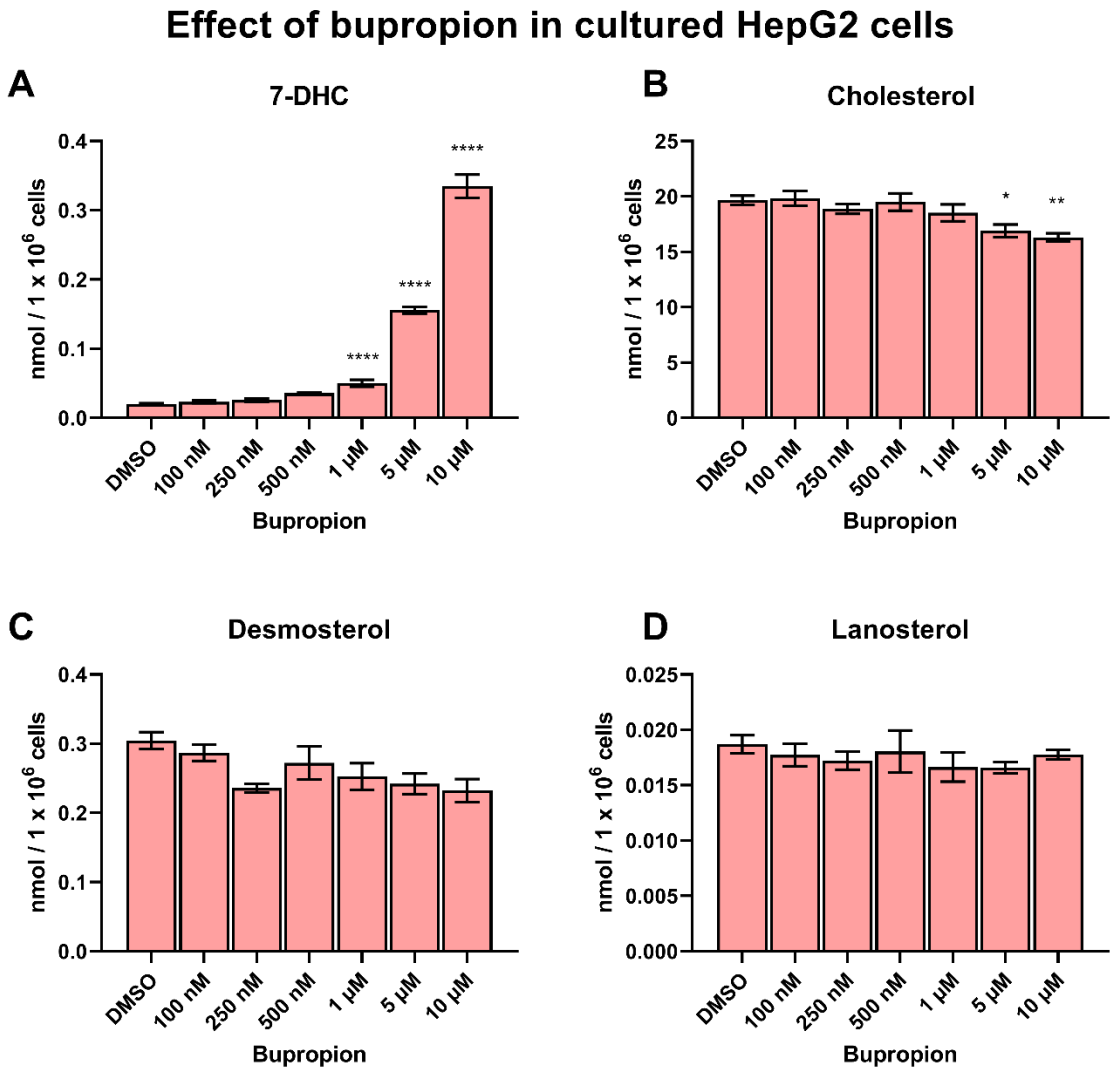
Supplemental Figure 1



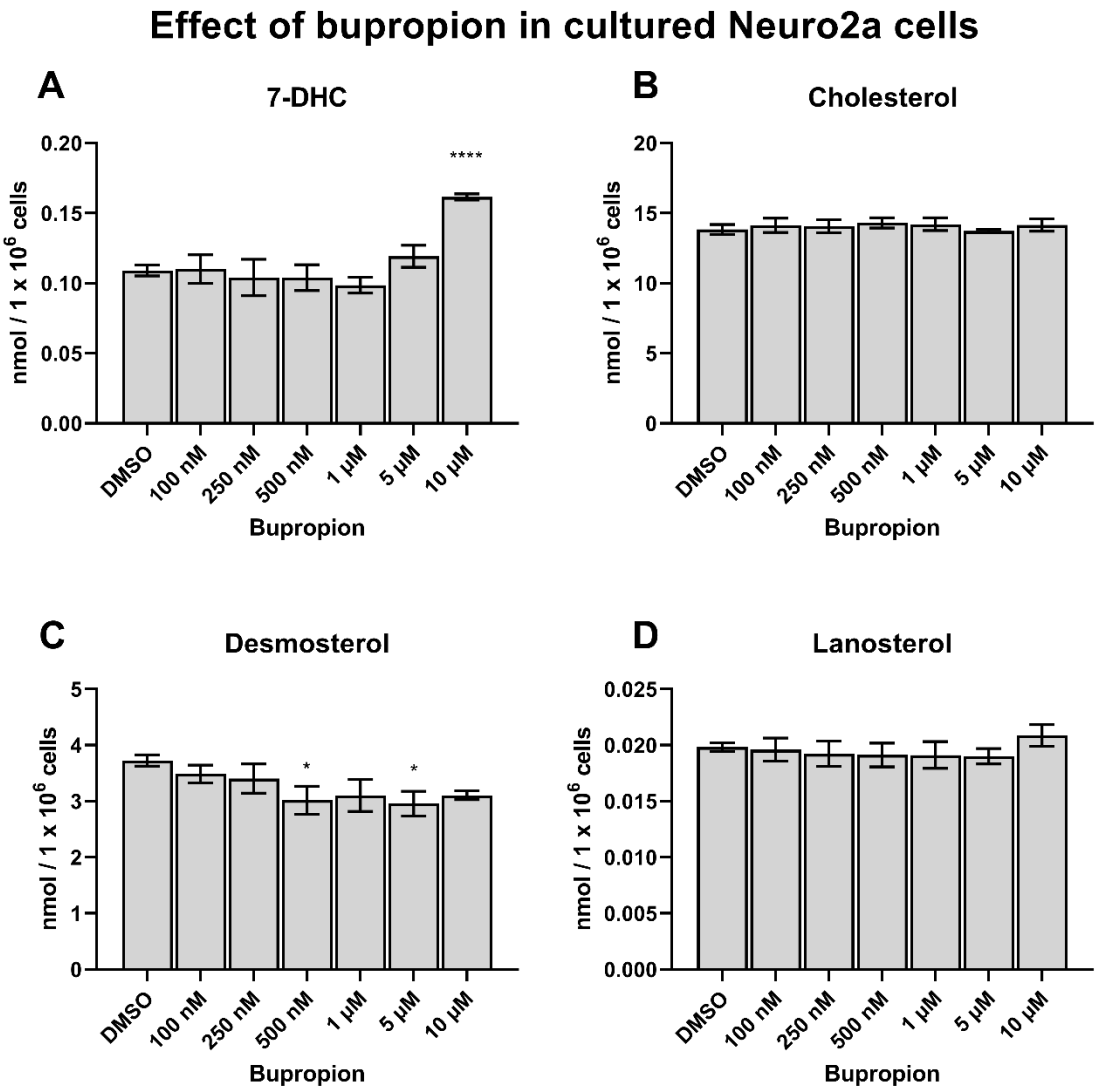
Supplemental Figure 1. **Distribution of desmosterol and lanosterol levels in serum of pregnant women and outlier identification. (A)** Levels of desmosterol and **(B)** lanosterol. Sterol levels are reported as ng/µL of serum. Each symbol corresponds to an individual sample. Red lines denote the upper 99% mean confidence of interval (CI).

Supplemental Figure 2



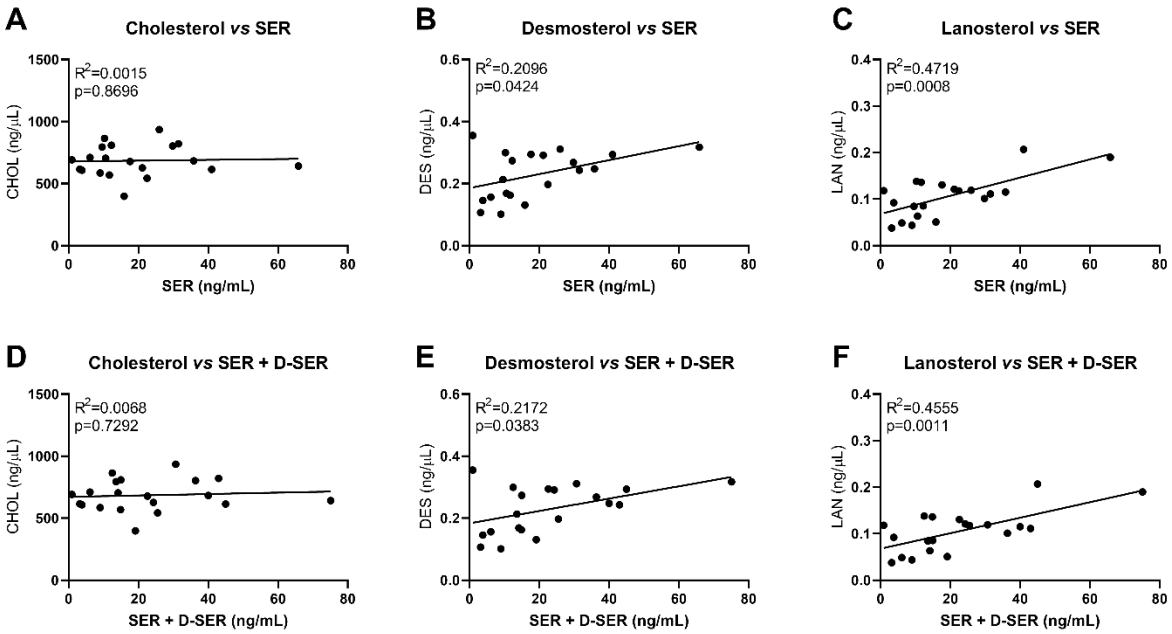
Supplemental Figure 2. **Sterol levels in cultured HepG2 cells treated with bupropion in a 96-well plate.** Cells were exposed to increasing concentrations of bupropion for 48 h and the sterol profile was determined using LC-MS/MS. Levels 7-DHC **(A)**, cholesterol **(B),** desmosterol **(C)** and lanosterol **(D)** were normalized using cell count (number of cells/well). Sterols are reported as nmol/million cells and the bars correspond to the mean ± SEM of six replicates. One-way ANOVA and Tukey's multiple comparisons tests were performed to compare difference between each treated group and the DMSO condition (control); statistical significance: *p<0.05; **p<0.01; ****p<0.0001

Supplemental Figure 3



<u>Supplemental Figure 3</u>. **Sterol levels in cultured Neuro2a cells treated with bupropion in a 96-well plate.** Cells were exposed to increasing concentrations of bupropion for 48 h and the sterol profile was determined using LC-MS/MS. Levels 7-DHC **(A)**, cholesterol **(B),** desmosterol **(C)** and lanosterol **(D)** were normalized using cell count (number of cells/well). Sterols are reported as nmol/million cells and the bars correspond to the mean ± SEM of six replicates. One-way ANOVA and Tukey's multiple comparisons tests were performed to compare difference between each treated group and the DMSO condition (control); statistical significance: *$p<0.05$; ****$p<0.0001$.

Supplemental Figure 4



<u>Supplemental Figure 4.</u> Correlation between sertraline (and its active metabolite desmethyl-sertraline) and sterol intermediates in sera of pregnant women. No significant correlations were observed between CHOL and SER **(A)** and CHOL and SER + D-SER **(D)**. Significant correlations were observed between **(B)** SER and DES, **(C)** SER and LAN, **(E)** SER+D-SER and DES and **(F)** SER+D-SER and LAN in pregnant women with confirmed SER in their sera. Each symbol denotes an individual sample. Lines denote simple linear regressions. Pearson's coefficients (R^2) and p values were determined using GraphPad Prism 9.

Cell cultures

Human hepatocellular carcinoma HEPG2 cells, human embryonic kidney HEK-293 cells, and mouse neuroblastoma cell line Neuro2a, were purchased from ATCC (Rockville, MD). The human and mouse cell lines were maintained in EMEM supplemented with L-glutamine, 10% FBS, and puromycin at 37°C and 5% CO_2. Cells were subcultured once a week and the culture medium changed every 2 days. For experimental purposes, the cells were plated in 96-well plates (for cell viability and sterol analysis). To assess the endogenous sterol synthesis, these cultures were grown in defined medium without cholesterol and without lipids by using EMEM with N2 supplement, L-glutamine, and puromycin. At the endpoint of the incubation, Hoechst dye was added to all wells in the 96-well plate and the total number of cells counted using an ImageXpress Pico and cell counting algorithm in CellReporterXpress. After removing the medium, wells were rinsed twice with 1X PBS and then stored at -80°C for lipid analysis. All samples were analyzed within 2 weeks of freezing. Sterol levels were analyzed in individual wells of 96-well plate and, for most experiments, cellular levels correspond to 8-12 technical replicates. After rinsing plates with 1X PBS (or removing previously frozen plates from -80°C freezer) 200 µL of MeOH containing the internal standard cocktail was added. The plate was placed on an orbital shaker for 30 min at room temperature. An aliquot (100 µL) of the supernatant was transferred to a PTAD-predeposited plate, sealed with Easy Pierce Heat Sealing Foil followed by 30 min agitation at room temperature, and analyzed by LC-MS/MS (as described in a following section). Values were normalized by average cell count and reported as nmol sterol/$1x10^6$ cells.