**Project:** NEFA Profile Response to Triphenyl Phosphate Exposure

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**NEFA Analysis:** William Keyes

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**Report Generation:** Michael La Frano (11/10/2015)

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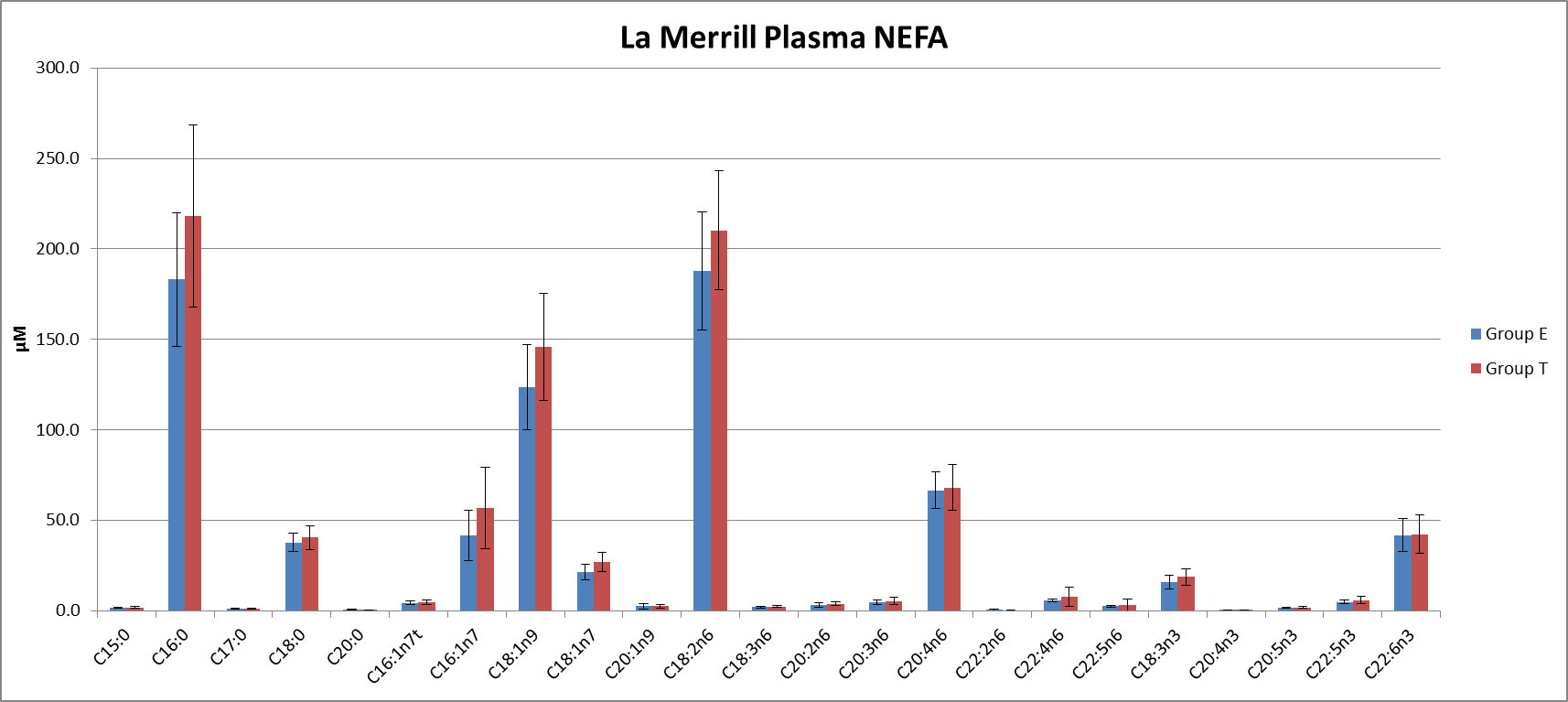
**Summary –** This study aims to identify changes in non-esterified fatty acid (NEFAs) in the plasma with triphenyl phosphate (TPP) exposure. UC Davis type 2 diabetes mellitus (UCD-T2DM) rats were treated with TPP or not treated. Each group was analyzed for non-esterified fatty acid (NEFA) changes to investigate alterations in NEFAs due to TPP exposure. Targeted analysis of NEFA in rat plasma samples was performed by the Newman lab.

**Important notes** regarding the samples included a high degree of hemolysis exhibited in the plasma. One sample was lost during processing (Group E- Subject 78). Two samples were outliers for multiple analytes and were not included in the final data (E-117 & T-28). Of the samples reported in this data set, there were no missing values.

Analytical results generally met quality control criterion with respect to surrogate recoveries and replicate precision. Surrogate recoveries (96%) and precision (11%RSD) were good for NEFAs. Analytical precision was assessed by analysis of 4 UTAK plasma samples. Analytical precision was acceptable, with %RSDs for analytes above 1 µM averaging 24% for the 4 Utaks and 14% for their compositions.

All data, including concentrations and relative abundance can be viewed in the attached **Final Data – La Merrill NEFA TPP** Excel file. An analyte list is also included. A general overview of the data can be seen in the Figures on pages 2-3. NEFA concentrations for each of the groups can be seen in **Figure 1** and relative abundance in **Figure 2**. A cursory statistical analysis of log transformed data of the individual fatty acids was performed in R using the Devium platform. Significance was determined using a two-sided t-test or Mann-Whitney U test depending on individual analyte normality. Results can be viewed in the Final Data – La Merrill NEFA TPP Excel file. Briefly, C16:1n7, C18:1n9, C18:1n7, and total NEFAs (P<0.04) all had significantly higher values in the “T” group. Palmitate (C16:0) was at the level of significance (P=0.05). In regards to relative abundance, the “E” group had a higher % of C20:4n6 but lower C18:1n7.

The final page contains a manuscript-ready description of the methods.

**Figure 1.** NEFA concentrations in groups (**µM ± STDEV**)

b

b

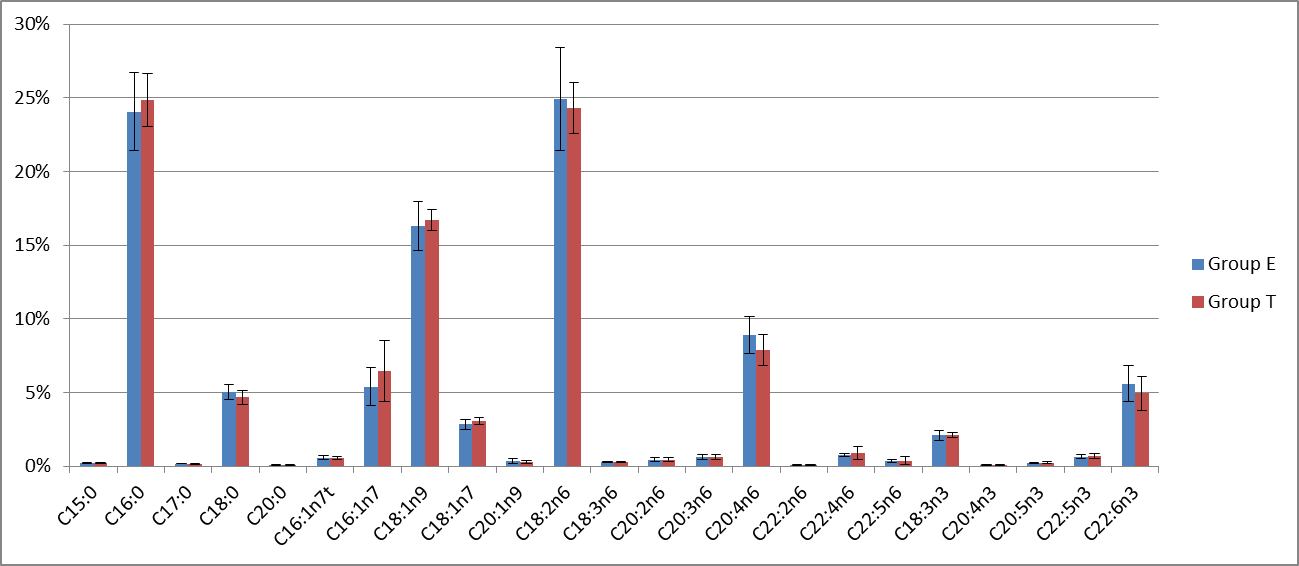
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**Figure 2.** Relative abundance of NEFAs (**%** **± STDEV**)



b

b

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**NEFA Extraction & Analysis**

Plasma non-esterified fatty acids (NEFAs) were isolated as previously described by Smedes (1). and Gladine et al. (2). Specifically, plasma aliquots (100 mL) were enriched with 5 mL 0.2 mg/ml butylated hydroxytoluene/EDTA in 1:1 methanol:- water, and a suite of extraction surrogates, which included deuterated-tri-palmitoyl glycerol (d31-16:0-TG; CDN Isotopes, Pointe-Claire, Quebec, Canada), deuterated distearoylphosphotidylcholine (d35-18:0-PC; Avanti Polar Lipids, Alabaster, Alabama), dodeca-(9E)-enoyl cholesterylesters (22:1n9-CE; NuChek Prep, Elysian MN) and dodecatrienoic acid (22:3n3; NuChek Prep). Lipids were then extracted with 10:8:11 cylcohexane: 2- propanol:ammonium acetate. Briefly, enriched samples were mixed with cyclopropane/2-propanol, phases were split with ammonium acetate, the organic phase was isolated and the aqueous phase was re-extracted with cyclohexane. The combined organic total lipid extract was reduced to dryness and reconstituted in 200 µL of 1:1 methanol/toluene and the total lipid extract was used to quantify plasma fatty acids as methyl esters by gas chromatography-mass spectrometry (GC-MS). It was derivitized by adding 45 μL 2M (trimethylsilyl) diazomethane in hexanes and spiked with 15:1n5 free acid to track methylation efficiency. Next, it was brought to a final volume of 200 mL with 90:10 methanol/toluene (v/v) and left at room temperature for 30 min, before being brought to dryness. The remaining fatty acid methyl esters (FAMEs) were re-constituted in 300 mL Hexane plus 10 uL of 44 mM tricosanoate methyl ester (23:0; NuChek Prep), vortexed, and 100 uL was transferred to a GC-MS Vial for analysis. A 1 µL injection was analyzed by GC-MS on an Agilent 6890/5973N MSD (Agilent Technologies, San Jose, CA) with electron impact ionization and in simultaneous selected ion monitoring/full scan mode. Analytes were separated on a 30 m x 0.25mm x 0.5µm DB-225 ms. Analytes were quantified with ChemStation vE.02.14 (Agilent Technologies) using internal standard methodologies against a 5 to 8 pt calibration curves.

(1) Smedes F (1999). Determination of total lipid using non-chlorinated solvents. Analyst. 124:1711-1718.

(2) Gladine et al (2014). Lipid profiling following intake of the omega 3 fatty acid DHA identifies peroxidized metabolites F4-neuroprostanes as the best predictors of atherosclerosis prevention. PLOS ONE.