# Can plasma lipidomics approaches be used to identify blood samples collected from sleep-deprived individuals?

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## BACKGROUND

- Sleep deprivation disrupts metabolic function, increases cellular stress, and taxes the body's immune system.
- Metabolite levels in plasma are regulated by the circadian clock and are modulated by sleep loss (Kasukawa et al., PNAS, 2012; Dallmann et al., PNAS, 2013; Davies et al., PNAS, 2014).
- Many lipid species exhibit strong circadian variation or increase/decrease during exposure to total sleep deprivation (Chua et al., PNAS, 2013; Chua et al., submitted).

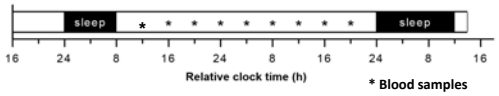
## HYPOTHESIS

***Lipid biomarkers can be used to distinguish blood samples collected during rested wakefulness versus sleep deprivation.***

## METHODS

- Subjects: 20 healthy males aged 21-28 years with regular sleep habits for at least one week (8 h time in bed per night).
- Intervention: Exposure to 40 hours of total sleep deprivation using constant routine procedures. Blood drawn every 4 hours.
- Lipidomics: Blood analyzed using mass spectrometry techniques.

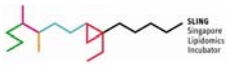
**40 hours of wakefulness**



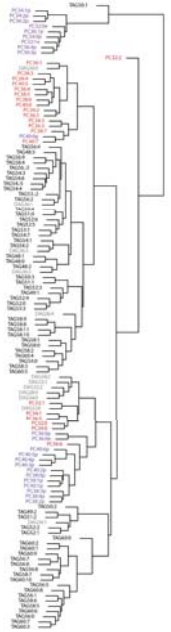

**Chronobiology laboratory**

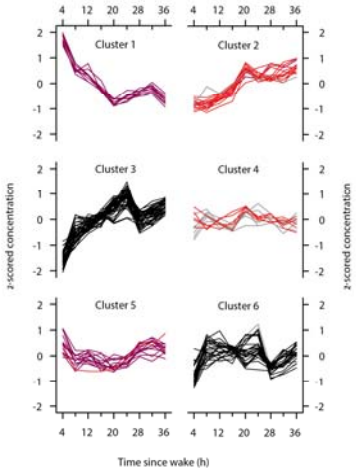
**Hourly snacks**

**Lipidomics (263 species)**




## 1. IDENTIFICATION OF LIPID CLUSTERS THAT VARY OVER TIME

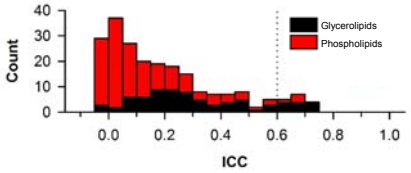





Agglomerative clustering was performed to identify groups of lipids that exhibited a similar time course. Three out of six clusters were validated as distinct clusters using the Davies-Bouldin Index and Silhouette criterion for clustering:

*Cluster 1*: choline plasmalogen species
*Cluster 2*: diacyl phosphatidylcholine species
*Cluster 3*: triglyceride and diglyceride species

## 2. LIPIDS WITH MOST RELIABLE TIME-OF-DAY VARIATION

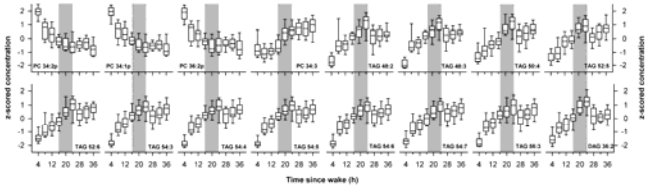



16 lipids with ICC > 0.6:
*Cluster 1*: 3 species
*Cluster 2*: 1 species
*Cluster 3*: 12 species

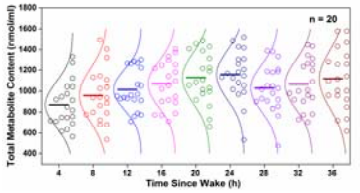
Higher intra-class correlation coefficients (ICC) indicate stronger time-of-day variation (within-subject differences) in concentrations relative to between-subject differences.

## 3. PREDICTING BLOOD SAMPLES DRAWN DURING SLEEP DEPRIVATION

**Lipids shortlisted for the maximum likelihood estimation (MLE) model**




**Mean and SD estimated for each lipid**




**MLE model performance**

| | RW | TSD |
|---|---|---|
| RW | TN 0.698 | FN 0.259 |
| TSD | FP 0.302 | TP 0.741 |

Twenty subjects were randomly partitioned into 2 groups of 10 subjects each, namely the reference group and test group. Maximum Likelihood Estimation (MLE) was applied to predict the time point at which given blood samples were drawn. For each shortlisted lipid, the mean and SD were estimated at each time point in the reference group. Model predictions were evaluated in the test group. Rested wakefulness (RW) was defined as <16 hours of wakefulness, and total sleep deprivation (TSD) was defined as ≥ 16 h of wakefulness. TN, true negative; TP, true positive; FP, false positive; FN, false negative.

## CONCLUSIONS

- Despite large between-subject differences in the concentrations of plasma lipids, a subset of metabolites showed reliable time-dependent variation during the sleep deprivation procedure.
- Under laboratory conditions, lipids could be used to identify blood samples collected during sleep deprivation better than chance (74% sensitivity, 70% specificity).
- We provide proof-of-concept that metabolomics based approaches can potentially be used to establish a bio-molecular signature of sleep loss.

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