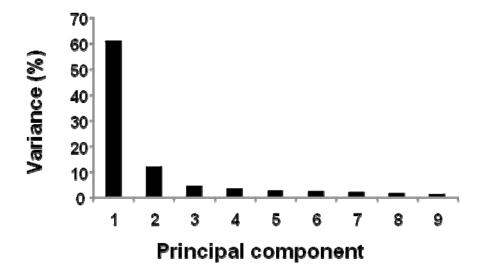
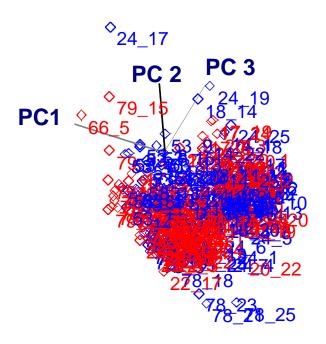
Appendix 1. Clinical data for human subjects

| Sex | Age | Height | Weight | BMI  | Fasting | BUN     | Serum      |
|-----|-----|--------|--------|------|---------|---------|------------|
|     |     | (m)    | (kg)   |      | glucose | (mg/dL) | creatinine |
|     |     |        |        |      | (mg/dL) |         | (mg/dL)    |
| F   | 22  | 1.57   | 63.5   | 25.6 | 69      | 8       | 0.9        |
| M   | 23  | 1.86   | 95.2   | 27.5 | 70      | 7       | 1.2        |
| M   | 25  | 1.80   | 71.8   | 22.2 | 72      | 14      | 0.9        |
| F   | 31  | 1.72   | 66.0   | 22.4 | 81      | 11      | 0.8        |
| F   | 45  | 1.53   | 57.9   | 24.6 | 86      | 10      | 0.8        |
| M   | 75  | 1.63   | 72.4   | 27.3 | 95      | 16      | 0.7        |
| M   | 79  | 1.64   | 87.6   | 32.6 | 90      | 25      | 0.9        |
| M   | 81  | 1.73   | 83.9   | 28.2 | 85      | 20      | 1.2        |
| F   | 82  | 1.48   | 44.2   | 20.2 | 82      | 9       | 0.7        |
| F   | 83  | 1.59   | 69.5   | 27.6 | 92      | 21      | 1.2        |

**Appendix 2. Percentage of variance explained by each of the first 9 Principal Components (PC).** Principal Component Analysis (PCA) was performed on 250 spectra from 25 hourly plasma samples collected over a 24-h period from 10 health individuals (5 males and 5 females) with 5 subjects <46 and 5 subjects >46 y. The first 3 PC included 77% of the total variation and PC7 contributed 1.7% of the total variation.

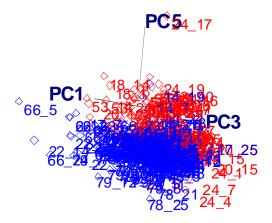


**Appendix 3. Little separation of plasma metabolic profiles of females and males was apparent by PCA.** Twenty-five hourly plasma samples were collected over a 24-h period from 10 health individuals (5 males and 5 females) with 5 subjects <46 and 5 subjects >46 y. Data were analyzed by unsupervised PCA and color-coded according to sex, where red represents males and blue represents females. A 3-dimensional Score Plot of PC1-PC3 revealed little separation according to sex.



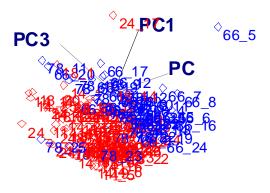
## Appendix 4. Partial separation of plasma metabolic profiles according to BMI as revealed by 3-D score plot of PCA.

Data were analyzed by unsupervised PCA and color coded into two groups, normal BMI (red, BMI 18.5-24.9) and overweight/obese (blue, BMI >25).



## Appendix 5. Partial separation of plasma metabolic profiles according to menopause depicted by 3-D score plot of PCA.

Data were analyzed by an unsupervised PCA and color-coded as premenopaual or post-menopausal. Among 5 female individuals, 3 were in the pre-menopause group, coded red, and 2 in the post-menopause group, coded blue.



Appendix 6 The *k*-means clustering results for individual subjects using k=3, with M representing the morning class, A representing the afternoon class and N representing the night class. Data underlined in bold font were considered afternoon class for the purpose of characterizing phase and duration of afternoon class. Criteria were that time points were considered in the afternoon sequence if A time point was not separated from the predominant A sequence by more than one non-A time point.

|       | Subject                             |   |  |                                       |  |  |  |   |              |                           |   |  |
|-------|-------------------------------------|---|--|---------------------------------------|--|--|--|---|--------------|---------------------------|---|--|
| Time  | 1                                   | 2   | 3  | 4                                     | 5  | 6  | 7  | 8   | 9            | 10                        | Majority Class<br>from k-means<br>clustering<br>in each subject | k-means on average   |
| 8:30  | M                                   | M   | M  | A                                     | N  | M  | M  | M   | N            | A                         | M   | M  |
| 9:30  | M                                   | M   | M  | M                                     | <u>A</u>   | N  | A  | M   | M            | A                         | M   | M  |
| 10:30 | A                                   | M   | A  | M                                     | N  | N  | M  | M   | M            | M                         | M   | M  |
| 11:30 | M                                   | $\underline{\mathbf{A}}$  | M  | M                                     | N  | M  | M  | M   | M            | M                         | M   | M  |
| 12:30 | N                                   | $\underline{\mathbf{M}}$  | M  | M                                     | N  | M  | $\underline{\mathbf{A}}$                                       | M   | M            | M                         | M   | M  |
| 13:30 | N                                   | <u>A</u>  | M  | M                                     | M  | <u>A</u>   | <u>A</u>   | <u>A</u>  | M            | <u>A</u>                  | $\underline{\mathbf{A}}$  | $\underline{\mathbf{A}}$   |
| 14:30 | Α                                   |   | <u>A</u>   | <u>A</u>                              | N  | <u>A</u> <u>N</u> <u>A</u> <u>N</u> <u>A</u> <u>A</u> <u>A</u> | <u>A</u> <u>A</u> <u>M</u> <u>A</u> <u>M</u> <u>A</u> <u>M</u> |   | N            |                           | A<br>A<br>M<br>A<br>A<br>A<br>M<br>A<br>M                       | <u>A</u> <u>A</u> <u>A</u> <u>A</u> <u>A</u> <u>A</u> <u>A</u> <u>A</u> <u>A</u> <u>M</u> <u>A</u> N |
| 15:30 | A                                   | <u>A</u>  | <u>A</u>   | <u>A</u>                              | N  | <u>A</u>   | <u>A</u>   | $\underline{\mathbf{M}}$  | A            | <u>A</u><br><u>A</u><br>N | <u>A</u>  | $\underline{\mathbf{A}}$   |
| 16:30 | N                                   | <u>A</u> <u>A</u> <u>A</u> <u>M</u> <u>A</u> <u>M</u> <u>A</u> <u>A</u> | <u>A</u> <u>A</u> <u>A</u> <u>A</u> <u>A</u> <u>A</u> <u>M</u> | <u>A</u> <u>A</u> <u>M</u> <u>A</u> N | M  | <u>N</u>   | $\underline{\mathbf{M}}$                                       | <u>A</u> <u>M</u> <u>A</u> <u>N</u> <u>A</u> <u>N</u> <u>A</u> <u>N</u> | M            |                           | $\underline{\mathbf{M}}$  | $\underline{\mathbf{A}}$   |
| 17:30 | M                                   | <u>A</u>  | <u>A</u>   | <u>A</u>                              | A  | <u>A</u>   | <u>A</u>   | <u>N</u>  | M            | N                         | <u>A</u>  | $\underline{\mathbf{A}}$   |
| 18:30 | M                                   | $\mathbf{M}$  | <u>A</u>   |                                       | A  |  | $\mathbf{M}$   | <u>A</u>  | A            | N                         | <u>A</u>  | <u>A</u>   |
| 19:30 | <u>A</u>                            | <u>A</u>  | <u>A</u>   | N                                     | M  | M  | <u>A</u>   | <u>N</u>  | N            | A                         | <u>A</u>  | <u>A</u>   |
| 20:30 | <u>A</u> <u>A</u> <u>A</u> <u>N</u> | $\mathbf{M}$  | $\mathbf{M}$   | A                                     | A  | M  | M  |   | M            | N                         | <u>A</u>  | <u>A</u>   |
| 21:30 | <u>A</u>                            | <u>A</u>  | $\underline{\mathbf{M}}$                                       | M                                     | M  | M  | M  | $\mathbf{M}$  | M            | A                         | $\underline{\mathbf{M}}$  | $\underline{\mathbf{M}}$   |
| 22:30 | <u>A</u>                            | <u>A</u>  | <u>M</u> <u>A</u> <u>A</u> <u>A</u>                            | M                                     | M  | M  | M  | <u>A</u>  | A            | N                         | $\underline{\mathbf{A}}$  | $\underline{\mathbf{A}}$   |
| 23:30 | <u>N</u>                            | N   | <u>A</u>   | M                                     | <u>A</u>   | A  | M  | M   | N            | N                         | N   |  |
| 0:30  | <u>A</u>                            | N   | <u>A</u>   | N                                     | <u>A</u>   | M  | N  | M   | N            | N                         | N   | N  |
| 1:30  | M                                   | N   | <u>A</u>   | N                                     | <u>A</u> <u>A</u> <u>A</u> <u>A</u> <u>A</u> <u>A</u> <u>A</u> | M  | M  | M   | N            | N                         | N   | N  |
| 2:30  | M                                   | N   | M  | N                                     | <u>A</u>   | N  | M  | A   | N            | N                         | N   | N  |
| 3:30  | N                                   | N   | M  | M                                     | <u>A</u>   | N  | M  | N   | A            | N                         | N   | N  |
| 4:30  | N                                   | N   | N  | N                                     | <u>A</u>   | M  | N  | M   | M            | N                         | N   | N  |
| 5:30  | N                                   | N   | N  | N                                     | <u>A</u>   | N  | N  | M   | N            | N                         | N   | N  |
| 6:30  | N                                   | M   | N  | M                                     |  | M  | N  | N   | M            | N                         | N   | N  |
| 7:30  | $\mathbf{M}$                        | N   | $\mathbf{M}$   | Α                                     | <u>A</u>   | $\mathbf{M}$   | N  | $\mathbf{M}$  | $\mathbf{M}$ | Α                         | M   | M  |