Katherine Hurst: Preliminary Report- Linear Mixed Model

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Introduction:

The aim of this project was to do the following

1: summarize and visualize the data

2: Fit a linear mixed effect model to the data comparing simulated and unstimulated saliva and adjust for age, bmi, gender and ethnicity if significant

3: Produce residual plots for each fitted model to check normality

4 : Test the effect between subject and within subject variability

5: perform correlation analysis on the other salivary parameters that were measured

PART I

# Data Pre-processing

The dataset was imported and prepared for analysis using R version 4.0. There were missing values for three variables which were not replaced as the numbers were small. Imputation is a possible consideration.

# Summary statistics:

Mean of Salivary parameters for SS and US

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| group | meanFR | sdFR | meanAge | sdAge | meanBMI | sdBMI | meanPro | sdPro | meanNa | sdNa | pop |
| US | 0.48 | 0.31 | 26.33 | 7.11 | 22.7 | 4.43 | 1.08 | 0.49 | 104.03 | 67.77 | 36 |
| SS | 1.20 | 0.58 | 26.33 | 7.11 | 22.7 | 4.43 | 0.96 | 0.36 | 193.58 | 147.02 | 36 |

Mean of Salivary parameters for SS and US con’t

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| group | Mean  Mg | Sd  Mg | Mean  P | Sd  P | Mean  S | Sd  S | Mean  K | Sd  K | Mean  Ca | Sd  Ca | Mean  Zn | Sd  Zn | pop |
| US | 3.66 | 2.212 | 159.24 | 54.43 | 34.96 | 20.220 | 760.62 | 190.69 | 51.60 | 12.54 | 9.68 | 33.13 | 36 |
| SS | 2.60 | 1.099 | 143.75 | 48.93 | 28.69 | 20.042 | 809.67 | 195.69 | 43.48 | 8.80 | 14.48 | 95.22 | 36 |

Mean of Salivary parameters for SS and US, by visit

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| group | visit | Mean  FR | Sd  FR | Mean  Age | Sd  Age | Mean  BMI | Sd  BMI | Mean  Pro | Sd  Pro | Mean  Na | Sd  Na | pop |
| US | V1 | 0.47 | 0.304 | 26.33 | 7.183 | 22.7 | 4.473 | 1.08 | 0.433 | 94.58 | 50.136 | 36 |
| US | V2 | 0.46 | 0.287 | 26.33 | 7.183 | 22.7 | 4.473 | 1.15 | 0.583 | 115.78 | 85.190 | 36 |
| US | V3 | 0.52 | 0.342 | 26.33 | 7.183 | 22.7 | 4.473 | 1.02 | 0.452 | 101.47 | 63.039 | 36 |
| SS | V2 | 1.21 | 0.621 | 26.33 | 7.183 | 22.7 | 4.473 | 1.04 | 0.442 | 200.05 | 134.939 | 36 |
| SS | V3 | 1.30 | 0.635 | 26.33 | 7.183 | 22.7 | 4.473 | 0.90 | 0.353 | 196.31 | 171.462 | 36 |
| SS | V4 | 1.10 | 0.500 | 26.33 | 7.183 | 22.7 | 4.473 | 0.94 | 0.274 | 184.38 | 135.431 | 36 |

Mean of Salivary parameters for SS and US, by visit con’t

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Group | visit | Mean  Mg | Sd  Mg | Mean  P | Sd  P | Mean  S | Sd  S | Mean  K | Sd  K | Mean  Ca | Sd  Ca | Mean  Zn | Sd  Zn | pop |
| US | V1 | 4.10 | 2.989 | 151.61 | 59.856 | 32.12 | 20.211 | 742.31 | 223.134 | 50.06 | 11.886 | 4.69 | 15.105 | 36 |
| US | V2 | 3.61 | 1.913 | 168.57 | 53.964 | 37.80 | 21.933 | 792.59 | 177.521 | 55.24 | 12.497 | 24.25 | 52.829 | 36 |
| US | V3 | 3.26 | 1.414 | 157.55 | 49.116 | 34.96 | 18.542 | 746.95 | 168.154 | 49.50 | 12.779 | 0.11 | 0.085 | 36 |
| SS | V2 | 2.54 | 1.298 | 147.04 | 43.494 | 29.76 | 24.580 | 825.34 | 171.293 | 45.04 | 8.842 | 43.23 | 162.624 | 36 |
| SS | V3 | 2.45 | 1.126 | 147.92 | 61.405 | 29.11 | 21.722 | 832.75 | 257.291 | 43.67 | 10.675 | 0.08 | 0.059 | 36 |
| SS | V4 | 2.81 | 0.819 | 136.28 | 39.644 | 27.19 | 12.177 | 770.93 | 138.533 | 41.74 | 6.278 | 0.12 | 0.066 | 36 |

Mean of Salivary parameters by subjects

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| patno | meanFR | sdFR | meanAge | sdAge | meanBMI | sdBMI | meanPro | sdPro | meanNa | sdNa |
| 1 | 1.03 | 0.851 | 31 | 0 | 22.58 | 0 | 0.80 | 0.189 | 164.69 | 69.303 |
| 2 | 0.48 | 0.084 | 20 | 0 | 17.80 | 0 | 0.50 | 0.135 | 102.52 | 25.481 |
| 4 | 1.05 | 0.277 | 40 | 0 | 16.53 | 0 | 0.95 | 0.130 | 116.47 | 66.350 |
| 5 | 1.23 | 0.556 | 25 | 0 | 21.56 | 0 | 0.75 | 0.200 | 110.90 | 37.325 |
| 6 | 1.04 | 0.521 | 36 | 0 | 29.74 | 0 | 1.01 | 0.216 | 254.39 | 54.569 |
| 7 | 0.34 | 0.264 | 26 | 0 | NaN | NA | 0.74 | 0.216 | 131.14 | 24.736 |
| 8 | 0.96 | 0.843 | 29 | 0 | 21.76 | 0 | 1.37 | 0.326 | 92.92 | 51.963 |
| 9 | 1.19 | 0.250 | 21 | 0 | 23.13 | 0 | 0.84 | 0.211 | 59.70 | 9.127 |
| 10 | 0.59 | 0.367 | 23 | 0 | 19.21 | 0 | 0.75 | 0.240 | 130.31 | 96.062 |
| 11 | 1.69 | 0.706 | 26 | 0 | 22.18 | 0 | 1.46 | 0.379 | 152.31 | 74.603 |
| 13 | 1.01 | 0.422 | 28 | 0 | 26.26 | 0 | 1.01 | 0.155 | 72.47 | 23.156 |
| 14 | 0.44 | 0.302 | 25 | 0 | 23.89 | 0 | 0.99 | 0.148 | 191.96 | 41.800 |
| 15 | 0.81 | 0.489 | 30 | 0 | 21.95 | 0 | 0.97 | 0.202 | 68.60 | 37.508 |
| 16 | 0.82 | 0.172 | 47 | 0 | 22.60 | 0 | 1.07 | 0.092 | 84.33 | 21.118 |
| 19 | 1.05 | 0.502 | 37 | 0 | 21.80 | 0 | 1.01 | 0.455 | 91.72 | 42.267 |
| 20 | 0.52 | 0.351 | 29 | 0 | 20.31 | 0 | 1.00 | 0.347 | 97.98 | 75.743 |
| 21 | 0.77 | 0.451 | 33 | 0 | 23.51 | 0 | 0.71 | 0.284 | 117.13 | 67.829 |
| 22 | 0.45 | 0.312 | 26 | 0 | 22.14 | 0 | 1.37 | 0.724 | 108.84 | 57.290 |
| 24 | 0.48 | 0.259 | 42 | 0 | 44.20 | 0 | 1.87 | 0.429 | 104.96 | 21.642 |
| 26 | 0.44 | 0.228 | 26 | 0 | 21.62 | 0 | 1.29 | 0.325 | 76.08 | 29.323 |
| 27 | 0.96 | 0.562 | 22 | 0 | 21.36 | 0 | 0.66 | 0.127 | 213.43 | 84.282 |
| 29 | 0.54 | 0.248 | 27 | 0 | 19.57 | 0 | 0.96 | 0.225 | 464.80 | 333.298 |
| 30 | 0.81 | 0.381 | 22 | 0 | 20.91 | 0 | 0.78 | 0.181 | 206.15 | 55.245 |
| 33 | 1.03 | 0.780 | 22 | 0 | 21.68 | 0 | 1.35 | 0.499 | 114.72 | 59.689 |
| 35 | 1.48 | 0.699 | 23 | 0 | 22.94 | 0 | 0.81 | 0.305 | 222.76 | 90.923 |
| 36 | 1.68 | 0.609 | 19 | 0 | 21.51 | 0 | 0.71 | 0.137 | 120.04 | 30.473 |
| 38 | 0.43 | 0.236 | 21 | 0 | 23.31 | 0 | 0.76 | 0.168 | 166.89 | 24.790 |
| 41 | 1.22 | 0.675 | 20 | 0 | 20.31 | 0 | 0.63 | 0.069 | 104.79 | 23.175 |
| 42 | 0.61 | 0.495 | 19 | 0 | 21.86 | 0 | 1.37 | 0.375 | 80.69 | 49.290 |
| 45 | 1.05 | 0.810 | 21 | 0 | 24.20 | 0 | 0.99 | 0.379 | 405.85 | 275.035 |
| 46 | 0.99 | 0.498 | 20 | 0 | 22.56 | 0 | 0.66 | 0.092 | 183.31 | 114.175 |
| 47 | 0.52 | 0.416 | 20 | 0 | 21.26 | 0 | 1.54 | 0.402 | 240.04 | 117.402 |
| 49 | 0.11 | 0.046 | 20 | 0 | 21.64 | 0 | 1.81 | 0.663 | 70.30 | 20.234 |
| 50 | 1.38 | 0.758 | 19 | 0 | 18.29 | 0 | 1.28 | 0.801 | 239.86 | 127.102 |
| 51 | 0.37 | 0.218 | 19 | 0 | 27.06 | 0 | 1.41 | 0.295 | 111.50 | 44.823 |
| 52 | 0.68 | 0.430 | 34 | 0 | 23.42 | 0 | 1.08 | 0.184 | 83.66 | 10.392 |

Mean of Salivary parameters by subjects con’t

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| patno | Mean  Mg | sdMg | meanP | sdP | meanS | sdS | meanK | sdK | Mean  Ca | sdCa | meanZn | sdZn |
| 1 | 1.97 | 0.452 | 161.15 | 13.816 | 32.50 | 7.912 | 772.78 | 90.200 | 49.96 | 4.727 | 9.09 | 22.191 |
| 2 | 1.03 | 0.255 | 91.03 | 9.520 | 12.37 | 2.727 | 535.18 | 47.879 | 48.52 | 6.463 | 0.05 | 0.020 |
| 4 | 2.41 | 0.354 | 142.66 | 15.516 | 20.27 | 3.167 | 706.91 | 56.527 | 47.41 | 6.765 | 35.98 | 56.592 |
| 5 | 2.57 | 0.921 | 121.05 | 26.406 | 33.15 | 8.750 | 776.85 | 25.283 | 49.17 | 3.955 | 21.62 | 34.310 |
| 6 | 2.91 | 0.816 | 131.51 | 8.622 | 54.25 | 10.888 | 750.45 | 28.837 | 53.30 | 5.229 | 16.39 | 38.410 |
| 7 | 3.50 | 0.872 | 146.96 | 15.366 | 44.28 | 8.181 | 688.15 | 37.218 | 41.70 | 6.434 | 0.23 | 0.214 |
| 8 | 4.25 | 1.456 | 217.39 | 56.315 | 39.15 | 11.420 | 1044.46 | 133.193 | 55.51 | 9.186 | 0.14 | 0.056 |
| 9 | 1.77 | 0.279 | 145.36 | 7.376 | 15.44 | 2.886 | 779.78 | 49.845 | 45.34 | 4.519 | 19.43 | 30.032 |
| 10 | 1.63 | 0.640 | 108.38 | 14.397 | 19.85 | 3.504 | 712.27 | 89.320 | 37.68 | 3.049 | 0.08 | 0.057 |
| 11 | 1.74 | 0.602 | 112.91 | 11.163 | 10.33 | 4.247 | 684.95 | 47.741 | 52.91 | 7.814 | 15.11 | 20.105 |
| 13 | 4.66 | 3.097 | 135.49 | 27.440 | 27.68 | 5.699 | 718.52 | 121.339 | 48.45 | 7.332 | 0.06 | 0.062 |
| 14 | 3.25 | 0.586 | 100.52 | 22.144 | 20.63 | 4.358 | 625.42 | 139.538 | 33.58 | 6.707 | 7.71 | 12.846 |
| 15 | 2.89 | 0.713 | 203.80 | 37.644 | 42.26 | 10.527 | 904.30 | 73.664 | 48.69 | 6.687 | 8.51 | 20.729 |
| 16 | 2.55 | 0.561 | 159.23 | 21.576 | 35.71 | 9.968 | 799.71 | 41.703 | 33.03 | 4.727 | 0.08 | 0.050 |
| 19 | 3.28 | 0.857 | 103.07 | 12.574 | 18.81 | 4.839 | 693.42 | 98.825 | 73.46 | 13.211 | 202.03 | 389.043 |
| 20 | 3.02 | 0.326 | 142.38 | 15.669 | 30.24 | 3.351 | 683.57 | 60.829 | 50.72 | 13.028 | 0.10 | 0.075 |
| 21 | 5.79 | 4.499 | 163.16 | 63.022 | 39.62 | 19.635 | 736.58 | 212.313 | 59.72 | 13.878 | 0.06 | 0.051 |
| 22 | 3.28 | 0.833 | 254.21 | 48.708 | 25.22 | 4.668 | 1088.32 | 158.000 | 50.23 | 11.417 | 25.78 | 60.643 |
| 24 | 4.77 | 1.110 | 257.37 | 61.378 | 99.77 | 26.676 | 1342.62 | 204.234 | 51.23 | 6.929 | 0.17 | 0.098 |
| 26 | 2.87 | 0.470 | 221.86 | 42.007 | 42.94 | 6.605 | 884.63 | 173.756 | 44.12 | 10.100 | 0.06 | 0.040 |
| 27 | 4.67 | 2.881 | 137.93 | 38.893 | 30.72 | 11.191 | 817.76 | 113.448 | 47.56 | 5.438 | 0.10 | 0.060 |
| 29 | 2.95 | 1.133 | 146.28 | 18.548 | 47.39 | 14.357 | 684.06 | 62.596 | 36.00 | 3.873 | 0.06 | 0.046 |
| 30 | 2.29 | 0.605 | 113.80 | 7.418 | 18.96 | 8.437 | 786.96 | 24.657 | 33.69 | 8.414 | 12.13 | 29.474 |
| 33 | 3.79 | 1.332 | 150.48 | 31.335 | 43.96 | 9.721 | 878.09 | 91.504 | 60.87 | 12.401 | 4.21 | 10.153 |
| 35 | 2.72 | 1.190 | 103.84 | 9.467 | 18.05 | 6.765 | 577.23 | 51.995 | 51.91 | 5.443 | 0.10 | 0.067 |
| 36 | 1.93 | 0.256 | 102.43 | 13.294 | 10.66 | 1.779 | 610.51 | 78.606 | 46.87 | 5.654 | 0.08 | 0.047 |
| 38 | 2.71 | 0.995 | 188.29 | 97.930 | 38.93 | 24.174 | 910.88 | 488.600 | 46.18 | 17.422 | 0.06 | 0.056 |
| 41 | 3.92 | 2.509 | 134.91 | 24.888 | 23.20 | 7.039 | 693.07 | 134.209 | 53.77 | 10.517 | 0.09 | 0.043 |
| 42 | 3.36 | 0.728 | 154.29 | 27.456 | 24.88 | 2.192 | 775.38 | 156.484 | 44.16 | 6.614 | 0.10 | 0.078 |
| 45 | 3.27 | 1.625 | 174.86 | 46.744 | 27.60 | 13.419 | 838.01 | 76.205 | 49.42 | 8.735 | 0.10 | 0.123 |
| 46 | 1.22 | 0.181 | 115.50 | 14.817 | 13.80 | 2.442 | 673.41 | 58.574 | 46.16 | 4.135 | 0.11 | 0.080 |
| 47 | 6.87 | 3.274 | 157.30 | 18.233 | 71.14 | 25.342 | 957.81 | 147.780 | 47.18 | 11.669 | 0.16 | 0.109 |
| 49 | 3.51 | 0.805 | 202.82 | 34.991 | 44.45 | 7.648 | 854.29 | 56.368 | 40.32 | 5.657 | 29.12 | 49.083 |
| 50 | 2.32 | 0.704 | 119.18 | 18.466 | 16.01 | 6.060 | 684.43 | 34.360 | 44.70 | 2.593 | 25.57 | 47.441 |
| 51 | 4.38 | 1.631 | 172.10 | 50.332 | 34.12 | 12.130 | 802.67 | 183.104 | 54.81 | 19.549 | 0.08 | 0.047 |
| 52 | 2.59 | 1.065 | 160.31 | 40.093 | 17.28 | 5.181 | 791.83 | 113.561 | 33.17 | 4.591 | 0.06 | 0.040 |

Repeated measurements by visit

|  |  |
| --- | --- |
| visit | count |
| V1 | 36 |
| V2 | 72 |
| V3 | 72 |
| V4 | 36 |

Gender statistics

|  |  |  |  |
| --- | --- | --- | --- |
| treatment | gender | N | pct |
| SS | Female | 29 | 81 |
| SS | Male | 7 | 19 |
| US | Female | 29 | 81 |
| US | Male | 7 | 19 |

Ethnicity statistics

|  |  |  |  |
| --- | --- | --- | --- |
| treatment | ethnicity | N | pct |
| SS | Asian | 9 | 25 |
| SS | Black | 1 | 3 |
| SS | Hispanic | 3 | 8 |
| SS | White | 23 | 64 |
| US | Asian | 9 | 25 |
| US | Black | 1 | 3 |
| US | Hispanic | 3 | 8 |
| US | White | 23 | 64 |

Variables with missing data

|  |  |  |  |
| --- | --- | --- | --- |
|  | sodiumconc | bmi | protenconc |
|  | 1 | 1 | 1 |
|  | 1 | 1 | 0 |
|  | 1 | 0 | 1 |
|  | 0 | 1 | 1 |
|  | 1 | 6 | 11 |

Visualization

Diagram, engineering drawing

Description automatically generated

![Chart

Description automatically generated]()

![Chart, line chart

Description automatically generated]()

Chart, scatter chart

Description automatically generated

Chart

Description automatically generated with medium confidence

![Calendar

Description automatically generated]()

**N.B More Visualizations could be produced for each outcome as specified**

**PART II: MIXED MODEL**

The dataset had repeated measurements made on each subject, each subject had multiple SS and US measurements of the outcome, which were likely to be correlated, i.e Flowrate (outcome of interest) are likely to be similar if they come from the same subject. The ICC(Intra class correlation) is used generally to measure how similar the outcome(flowrate in this case) is if they come from the same subject. This model adds a random effect for each subject which allowed a different baseline outcome measurement for each subject (accounting for individual differences) and also a random effect for visit (accounting for time differences)

**Mixed Model for Flowrate**

Bmi, age, gender, ethnicity were not statistically significant predictors. Mean flow rate for SS was higher compared to US and this was statistically significant. ICC of patno = 59% means that 59% of variation in flow rate was explained by differences in participants and only 1% explained by differences over the visits. This interpretation is the same for all models. Repeated measurements for each subject were highly correlated. There was hardly any correlation (similarity of flowrate measurements) over visits. Residuals were normally distributed and Shapiro-Wilk test also confirmed that (p >0.05). The between group effect was highly significant (SS/US) <0.001, while the within group effect was not statistically significant, 0.13. This result test the effect of the group factor and repeatability over visit. In cases where there is group effect, there is a shift on the combined mean for SS compared to US and within one group, the mean value over visits(v1,v1,v3,v4) could be observed for patterns or not

**Model coding; US = 0(refence group), SS = 1**

**Mean difference = difference of US and SS**

|  |  |  |  |
| --- | --- | --- | --- |
| Fixed effect | | | |
|  | **Mean difference** | **95%CI** | **P value** |
| **Treatment (SS)** | 0.74 | 0.66- 0.83 | <0.001 |
| Random effect | | | |
|  | **ICC** | **Standard deviation** | **Groups variables** |
| **Subject** | 0.59 | 0.36 | 36 |
| **Visit** | 0.01 | 0.06 | 4 |
| **Residual** |  | 0.29 |  |

|  |  |
| --- | --- |
| Between and within subject effect | |
|  | P value |
| visit | 0.13 |
| Treatment | <0.001 |

|  |  |
| --- | --- |
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|  |  |
| --- | --- |
|  | Shapiro-Wilk normality test: p-value = 0.08571 |

**Mixed Model for Protein Concentration**

Bmi, age, gender was not statistically significant. There was a significant difference between US and SS. However, residuals were not normally distributed. Many consider using gamma distribution later.

|  |  |  |  |
| --- | --- | --- | --- |
| Fixed effect | | | |
|  | **Mean difference** | **95%CI** | **P value** |
| **Treatment (SS)** | -0.11 | -0.21 : -0.02 | 0.02 |
| Random effect | | | |
|  | **ICC** | **Standard deviation** | **Groups variables** |
| **Subject** | 0.46 | 0.30 | 36 |
| **Visit** | 0.01 | 0.04 | 4 |
| **Residual** |  | 0.32 |  |

|  |  |
| --- | --- |
| Between and within subject effect | |
|  | P value |
| visit | 0.36 |
| Treatment | 0.01 |

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|  |  |
| --- | --- |
|  | Shapiro-Wilk normality test: p-value = <0.001 |

After deleting outliers and refitting the model, it failed to converge

**Mixed Model for Sodium Concentration**

Bmi, age, gender was not statistically significant. There was a significant difference between US and SS. Residual were not normally distributed. Outliers were detected and deleted, and model refitted, and residuals were still not normally distributed. Many consider using gamma distribution later

|  |  |  |  |
| --- | --- | --- | --- |
| Fixed effect | | | |
|  | **Mean difference** | **95%CI** | **P value** |
| **Treatment (SS)** | 89.91 | 68.73 : 111.10 | <0.00 |
| Random effect | | | |
|  | **ICC** | **Standard deviation** | **Groups variables** |
| **Subject** | 0.52 | 82.02 | 36 |
| **Visit** | 0.00 | 0.00 | 4 |
| **Residual** |  | 79.21 |  |

|  |  |
| --- | --- |
| Between and within subject effect | |
|  | P value |
| visit | -- |
| Treatment | <0.001 |

There was a very correlation between visit and subjects that model didn’t estimate, so visit was omitted

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**Mixed Model for Magnesium**

Bmi, age, gender was not statistically significant. There was a significant difference between US and SS Residual were not normally distributed.

|  |  |  |  |
| --- | --- | --- | --- |
| Fixed effect | | | |
|  | **Mean difference** | **95%CI** | **P value** |
| **Treatment (SS)** | -1.01 | -1.40 : -0.62 | <0.00 |
| Random effect | | | |
|  | **ICC** | **Standard deviation** | **Groups variables** |
| **Subject** | 0.39 | 1.02 | 36 |
| **Visit** | 0.02 | 0.22 | 4 |
| **Residual** |  | 1.35 |  |

|  |  |
| --- | --- |
| Between and within subject effect | |
|  | P value |
| visit | 0.2 |
| Treatment | <0.001 |

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**Mixed Model for Phosphorus**

Bmi, age, gender was not statistically significant. There was a significant difference between US and SS Residual were not normally distributed.

|  |  |  |  |
| --- | --- | --- | --- |
| Fixed effect | | | |
|  | **Mean difference** | **95%CI** | **P value** |
| **Treatment (SS)** | -15.52 | 25.07 : -5.98 | <0.00 |
| Random effect | | | |
|  | **ICC** | **Standard deviation** | **Groups variables** |
| **Subject** | 0.58 | 39.23 | 36 |
| **Visit** | 0.01 | 4.67 | 4 |
| **Residual** |  | 33.22 |  |

|  |  |
| --- | --- |
| Between and within subject effect | |
|  | P value |
| visit | 0.34 |
| Treatment | <0.001 |

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**Mixed Model for Potassium**

Bmi, age, gender was not statistically significant. There was a significant difference between US and SS . Residual were not normally distributed.

|  |  |  |  |
| --- | --- | --- | --- |
| Fixed effect | | | |
|  | **Mean difference** | **95%CI** | **P value** |
| **Treatment (SS)** | 52.44 | 15.14 : 89.75 | 0.01 |
| Random effect | | | |
|  | **ICC** | **Standard deviation** | **Groups variables** |
| **Subject** | 0.54 | 141.49 | 36 |
| **Visit** | 0.01 | 18.64 | 4 |
| **Residual** |  | 129.53 |  |

|  |  |
| --- | --- |
| Between and within subject effect | |
|  | P value |
| visit | 0.32 |
| Treatment | 0.006 |

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|  |

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| --- | --- |
|  |  |

**Mixed Model for Calcium**

Bmi, age, gender was not statistically significant. Residual were not normally distributed.

|  |  |  |  |
| --- | --- | --- | --- |
| Fixed effect | | | |
|  | **Mean difference** | **95%CI** | **P value** |
| **Treatment (SS)** | -8.08 | -10.30 : -5.82 | 0.00 |
| Random effect | | | |
|  | **ICC** | **Standard deviation** | **Groups variables** |
| **Subject** | 0.50 | 7.69 | 36 |
| **Visit** | 0.02 | 1.71 | 4 |
| **Residual** |  | 7.42 |  |

|  |  |
| --- | --- |
| Between and within subject effect | |
|  | P value |
| visit | 0.02 |
| Treatment | <0.001 |

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| --- | --- |
|  |  |

**Mixed Model for Zinc**

The effect Bmi, age, gender was not statistically significant. Residual were not normally distributed.

|  |  |  |  |
| --- | --- | --- | --- |
| Fixed effect | | | |
|  | **Mean difference** | **95%CI** | **P value** |
| **Treatment (SS)** | 6.98 | -12.81 : 26.77 | 0.49 |
| Random effect | | | |
|  | **ICC** | **Standard deviation** | **Groups variables** |
| **Subject** | 0.08 | 20.14 | 36 |
| **Visit** | 003 | 12.74 | 4 |
| **Residual** |  | 66.82 |  |

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**Mixed Model for Sulphur Concentration**

Bmi, age, gender was not statistically significant. There was a significant difference between US and SS. However, residuals were not normally distributed. Many consider using gamma distribution later.

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| --- | --- | --- | --- |
| Fixed effect | | | |
|  | **Mean difference** | **95%CI** | **P value** |
| **Treatment (SS)** | -6.5 | 9.44 : -3.55 | 0.00 |
| Random effect | | | |
|  | **ICC** | **Standard deviation** | **Groups variables** |
| **Subject** | 0.74 | 17.21 | 36 |
| **Visit** | 0.01 | 1.46 | 4 |
| **Residual** |  | 10.22 |  |

|  |  |
| --- | --- |
| Between and within subject effect | |
|  | P value |
| visit | 0.32 |
| Treatment | <0.001 |

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