Glutathione ratios as the mechanism of action of lipoic acid in progressive multiple sclerosis (PI: Rebecca Spain)

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# Import raw data

Joined data set. Include only the

* Study design variables,
* Normalized concentrations, and
* GSH/GSSG ratios

## Clean data

Data check **before cleaning**.

|  |  |  |  |
| --- | --- | --- | --- |
| patientID | hasM0 | hasM3 | hasM12 |
| 120 | 1 | 0 | 1 |
| 122 | 1 | 0 | 1 |
| 134 | 1 | 1 | 2 |
| 143 | 0 | 2 | 0 |

Recode data per Cassidy's email

From: Cassidy Taylor  
Sent: Monday, March 12, 2018 3:17 PM  
To: Benjamin Chan [chanb@ohsu.edu](mailto:chanb@ohsu.edu); Rebecca Spain [spainr@ohsu.edu](mailto:spainr@ohsu.edu)  
Subject: RE: Rebecca Spain Glutathione Study

Just heard back from the lab and based on the remaining levels of blood, the manifest was incorrectly labeled. It should read

Sample 44 = 143 M0  
Sample 45 = 143 M3  
Sample 46 = 143 M12

Data check **after cleaning**. Should return a data frame with only patientID %in% c(120, 122).

|  |  |  |  |
| --- | --- | --- | --- |
| patientID | hasM0 | hasM3 | hasM12 |
| 120 | 1 | 0 | 1 |
| 122 | 1 | 0 | 1 |

## Create analytic data frames for each aim

Aim 1

## Classes 'tbl\_df', 'tbl' and 'data.frame': 58 obs. of 9 variables:  
## $ sampleID : num 1 2 3 4 5 6 7 8 9 10 ...  
## $ patientID : Factor w/ 20 levels "118","119","120",..: 1 1 1 2 2 2 3 3 4 4 ...  
## $ visitMonth : Factor w/ 3 levels "0","3","12": 1 2 3 1 2 3 1 3 1 3 ...  
## $ studyArm : Factor w/ 2 levels "LA","Placebo": 1 1 1 2 2 2 2 2 2 2 ...  
## $ normalizedConcGSH : num 125 101 137 87 171 ...  
## $ normalizedConcGSSG : num 0.931 0.639 0.828 0.693 1.291 ...  
## $ normalizedConcStdEGSH : num 2.287 2.06 0.0847 0.3121 1.1748 ...  
## $ normalizedConcStdEGSSG: num 0.223 0.17 0.229 0.186 0.352 ...  
## $ normalizedConcRatio : num 134 158 166 126 132 ...

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| patientID | visitMonth | studyArm | normalizedConcGSH | normalizedConcGSSG | normalizedConcRatio |
| 118 | 0 | LA | 125.06 | 0.93 | 134.39 |
| 118 | 3 | LA | 100.76 | 0.64 | 157.79 |
| 118 | 12 | LA | 137.35 | 0.83 | 165.82 |
| 124 | 0 | LA | 127.61 | 0.76 | 168.90 |
| 124 | 3 | LA | 86.82 | 0.51 | 169.44 |
| 124 | 12 | LA | 109.10 | 0.64 | 171.58 |
| 129 | 0 | LA | 175.84 | 1.03 | 171.36 |
| 129 | 3 | LA | 134.91 | 0.96 | 140.36 |
| 129 | 12 | LA | 120.29 | 0.69 | 175.25 |
| 130 | 0 | LA | 102.18 | 0.69 | 148.02 |
| 130 | 3 | LA | 142.32 | 0.94 | 151.86 |
| 130 | 12 | LA | 170.98 | 0.97 | 176.26 |
| 132 | 0 | LA | 164.86 | 1.18 | 139.21 |
| 132 | 3 | LA | 159.88 | 0.95 | 168.87 |
| 132 | 12 | LA | 156.80 | 0.95 | 165.67 |
| 134 | 0 | LA | 121.36 | 0.86 | 140.53 |
| 134 | 3 | LA | 117.52 | 0.68 | 173.03 |
| 134 | 12 | LA | 166.19 | 0.94 | 176.27 |
| 140 | 0 | LA | 135.54 | 0.82 | 166.15 |
| 140 | 3 | LA | 123.01 | 0.81 | 152.21 |
| 140 | 12 | LA | 145.25 | 0.99 | 147.36 |
| 149 | 0 | LA | 111.48 | 0.71 | 157.75 |
| 149 | 3 | LA | 120.45 | 0.84 | 143.15 |
| 149 | 12 | LA | 133.68 | 0.83 | 160.44 |
| 151 | 0 | LA | 101.74 | 0.60 | 170.28 |
| 151 | 3 | LA | 92.33 | 0.58 | 160.44 |
| 151 | 12 | LA | 108.97 | 0.66 | 166.27 |
| 119 | 0 | Placebo | 86.95 | 0.69 | 125.56 |
| 119 | 3 | Placebo | 171.00 | 1.29 | 132.47 |
| 119 | 12 | Placebo | 185.79 | 1.42 | 131.09 |
| 120 | 0 | Placebo | 108.99 | 0.81 | 134.69 |
| 120 | 12 | Placebo | 144.20 | 1.00 | 143.82 |
| 122 | 0 | Placebo | 124.43 | 0.97 | 127.89 |
| 122 | 12 | Placebo | 126.22 | 0.94 | 134.53 |
| 123 | 0 | Placebo | 96.34 | 0.63 | 152.45 |
| 123 | 3 | Placebo | 131.28 | 1.00 | 131.92 |
| 123 | 12 | Placebo | 110.30 | 0.86 | 128.95 |
| 125 | 0 | Placebo | 93.27 | 0.74 | 126.19 |
| 125 | 3 | Placebo | 91.82 | 0.64 | 143.24 |
| 125 | 12 | Placebo | 116.12 | 0.90 | 128.79 |
| 131 | 0 | Placebo | 183.91 | 1.47 | 124.82 |
| 131 | 3 | Placebo | 129.58 | 1.02 | 126.79 |
| 131 | 12 | Placebo | 116.74 | 0.93 | 125.19 |
| 135 | 0 | Placebo | 109.98 | 0.71 | 155.24 |
| 135 | 3 | Placebo | 133.95 | 0.83 | 160.95 |
| 135 | 12 | Placebo | 143.38 | 0.97 | 148.21 |
| 139 | 0 | Placebo | 162.97 | 1.16 | 140.65 |
| 139 | 3 | Placebo | 156.30 | 1.14 | 137.41 |
| 139 | 12 | Placebo | 161.13 | 1.14 | 141.52 |
| 143 | 0 | Placebo | 143.55 | 1.14 | 125.84 |
| 143 | 3 | Placebo | 169.13 | 1.33 | 127.29 |
| 143 | 12 | Placebo | 97.65 | 0.55 | 176.13 |
| 145 | 0 | Placebo | 110.28 | 0.90 | 122.08 |
| 145 | 3 | Placebo | 97.01 | 0.78 | 123.97 |
| 145 | 12 | Placebo | 109.46 | 0.83 | 132.14 |
| 153 | 0 | Placebo | 100.72 | 0.69 | 146.84 |
| 153 | 3 | Placebo | 93.73 | 0.77 | 121.86 |
| 153 | 12 | Placebo | 96.02 | 0.80 | 120.52 |

Aim 2

## Classes 'tbl\_df', 'tbl' and 'data.frame': 20 obs. of 6 variables:  
## $ patientID : Factor w/ 20 levels "118","119","120",..: 1 2 3 4 5 6 7 8 9 10 ...  
## $ studyArm : Factor w/ 2 levels "LA","Placebo": 1 2 2 2 2 1 2 1 1 2 ...  
## $ concRatioM0 : num 134 126 135 128 152 ...  
## $ concRatioM12 : num 166 131 144 135 129 ...  
## $ pctChangeConcRatio: num 23.39 4.4 6.78 5.2 -15.41 ...  
## $ brainAtrophy : num 0.358 -2.026 -1.544 0.534 -0.679 ...

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| patientID | studyArm | concRatioM0 | concRatioM12 | pctChangeConcRatio | brainAtrophy |
| 118 | LA | 134.39 | 165.82 | 23.39 | 0.36 |
| 124 | LA | 168.90 | 171.58 | 1.59 | 0.80 |
| 129 | LA | 171.36 | 175.25 | 2.27 | -1.46 |
| 130 | LA | 148.02 | 176.26 | 19.08 | 0.20 |
| 132 | LA | 139.21 | 165.67 | 19.01 | -1.55 |
| 134 | LA | 140.53 | 176.27 | 25.44 | 0.60 |
| 140 | LA | 166.15 | 147.36 | -11.31 | -1.11 |
| 149 | LA | 157.75 | 160.44 | 1.71 | 0.07 |
| 151 | LA | 170.28 | 166.27 | -2.36 | -0.16 |
| 119 | Placebo | 125.56 | 131.09 | 4.40 | -2.03 |
| 120 | Placebo | 134.69 | 143.82 | 6.78 | -1.54 |
| 122 | Placebo | 127.89 | 134.53 | 5.20 | 0.53 |
| 123 | Placebo | 152.45 | 128.95 | -15.41 | -0.68 |
| 125 | Placebo | 126.19 | 128.79 | 2.06 | -1.12 |
| 131 | Placebo | 124.82 | 125.19 | 0.29 | -1.75 |
| 135 | Placebo | 155.24 | 148.21 | -4.52 | -0.27 |
| 139 | Placebo | 140.65 | 141.52 | 0.62 | -2.17 |
| 143 | Placebo | 125.84 | 176.13 | 39.96 | -1.74 |
| 145 | Placebo | 122.08 | 132.14 | 8.25 | -2.01 |
| 153 | Placebo | 146.84 | 120.52 | -17.93 | -1.22 |

# Aim 1

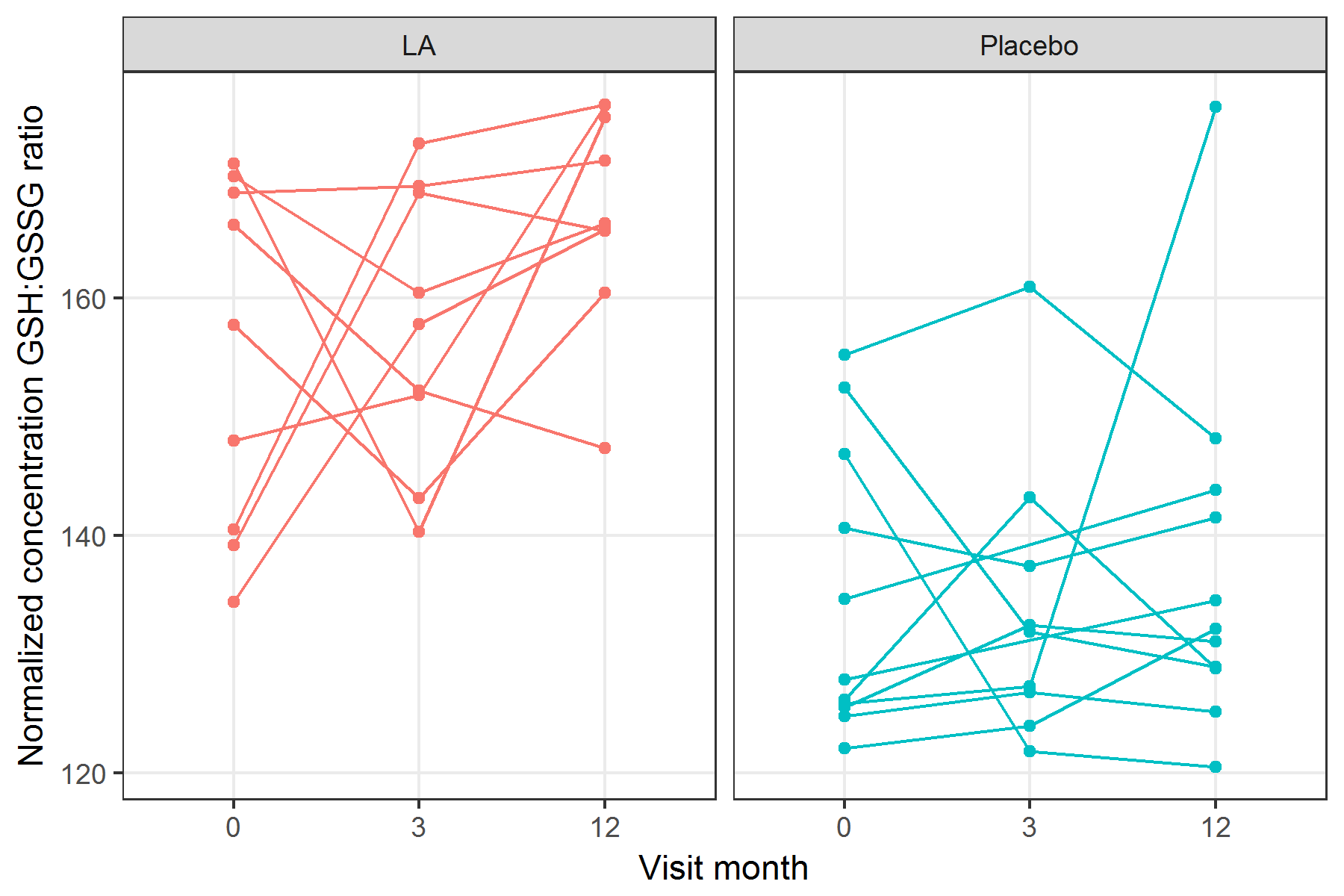
The model for Aim 1 will be a random intercept linear model. Estimation will use the *lme4* package.

##   
## To cite lme4 in publications use:  
##   
## Douglas Bates, Martin Maechler, Ben Bolker, Steve Walker (2015).  
## Fitting Linear Mixed-Effects Models Using lme4. Journal of  
## Statistical Software, 67(1), 1-48. doi:10.18637/jss.v067.i01.  
##   
## A BibTeX entry for LaTeX users is  
##   
## @Article{,  
## title = {Fitting Linear Mixed-Effects Models Using {lme4}},  
## author = {Douglas Bates and Martin M{\"a}chler and Ben Bolker and Steve Walker},  
## journal = {Journal of Statistical Software},  
## year = {2015},  
## volume = {67},  
## number = {1},  
## pages = {1--48},  
## doi = {10.18637/jss.v067.i01},  
## }

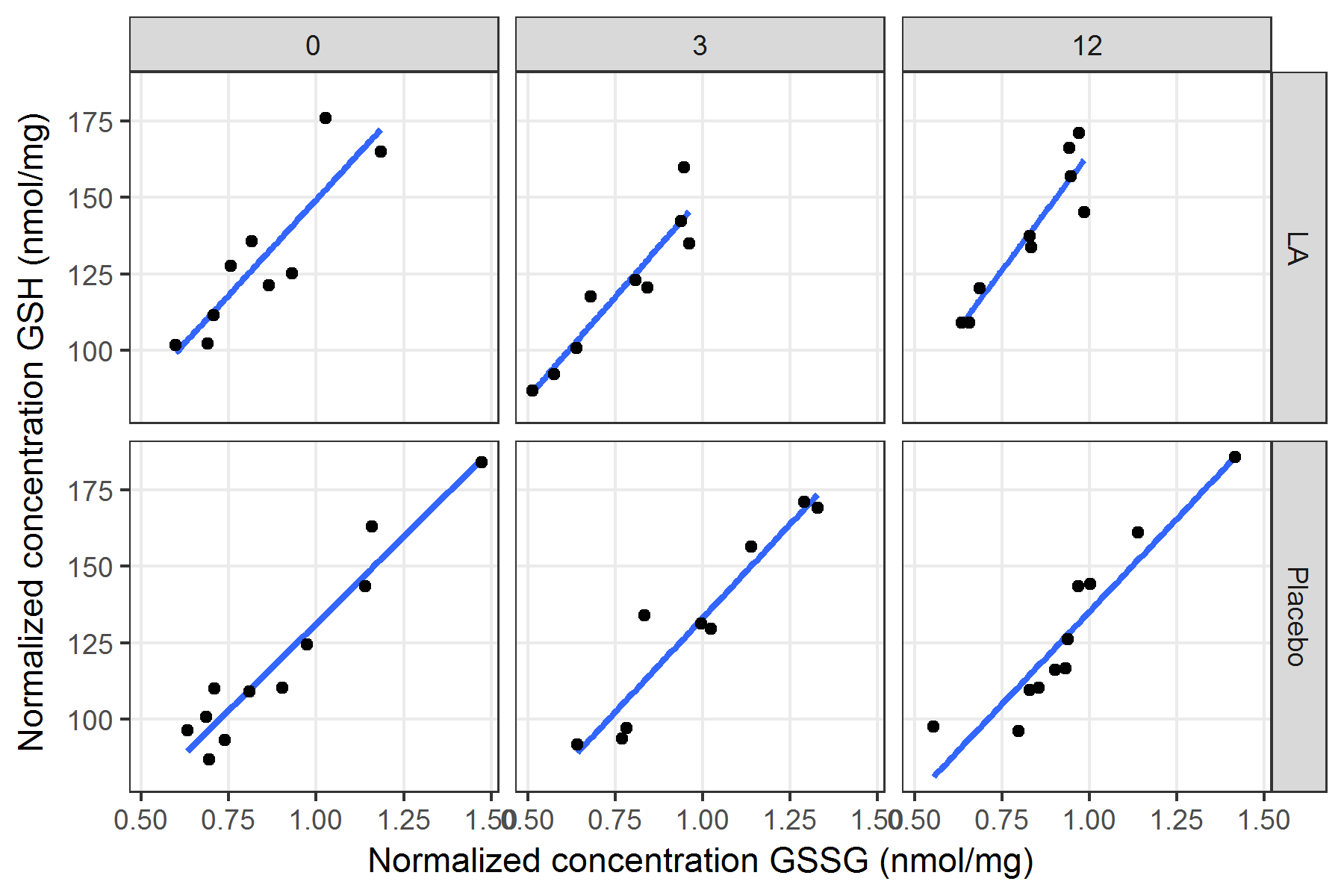
The model is

where is the random intercept component for each study subject , and is the random error.

## Normalized GSH:GSSG concentration ratio



figures/lineplotNormalizedConcRatio.png



figures/plotNormalizedConcSlope.png

Mixed effects model using the **lme4** package.

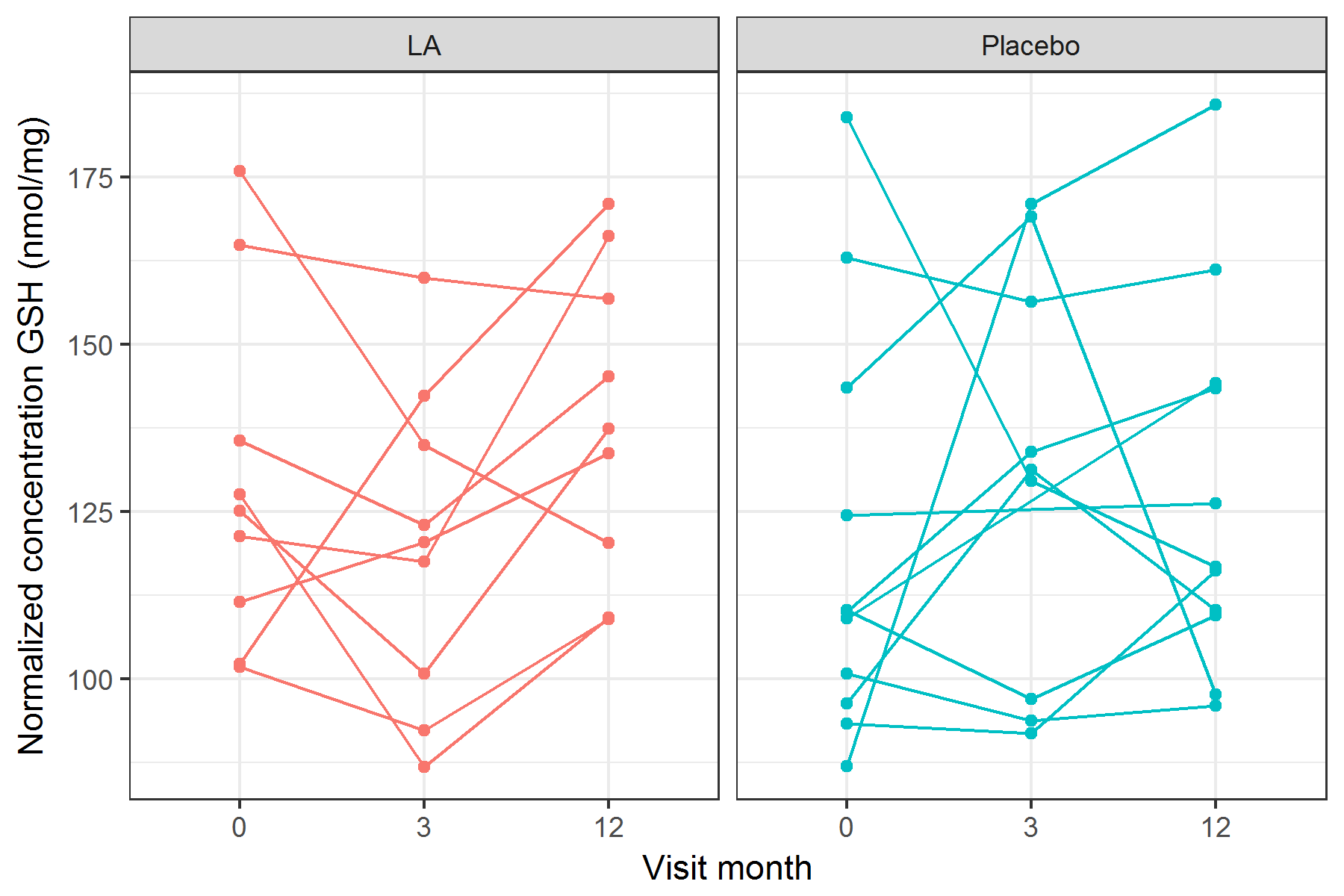
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | df | pvalue |
| (Intercept) | 155.18 | 4.26 | 36.42 | 34 | 0.0000 |
| studyArmPlacebo | -20.43 | 5.74 | -3.56 | 18 | 0.0023 |
| visitMonth3 | 2.29 | 5.94 | 0.38 | 34 | 0.7027 |
| visitMonth12 | 12.04 | 5.94 | 2.03 | 34 | 0.0505 |
| studyArmPlacebo:visitMonth3 | -3.05 | 8.21 | -0.37 | 34 | 0.7121 |
| studyArmPlacebo:visitMonth12 | -9.43 | 8.01 | -1.18 | 34 | 0.2469 |

Details (not shown).

Mixed effects model using the **nlme** package.

*Not executed.* The lme4::lmer() function is good enough.

## Normalized concentration GSH



figures/lineplotNormalizedConcGSH.png

Mixed effects model using the **lme4** package.

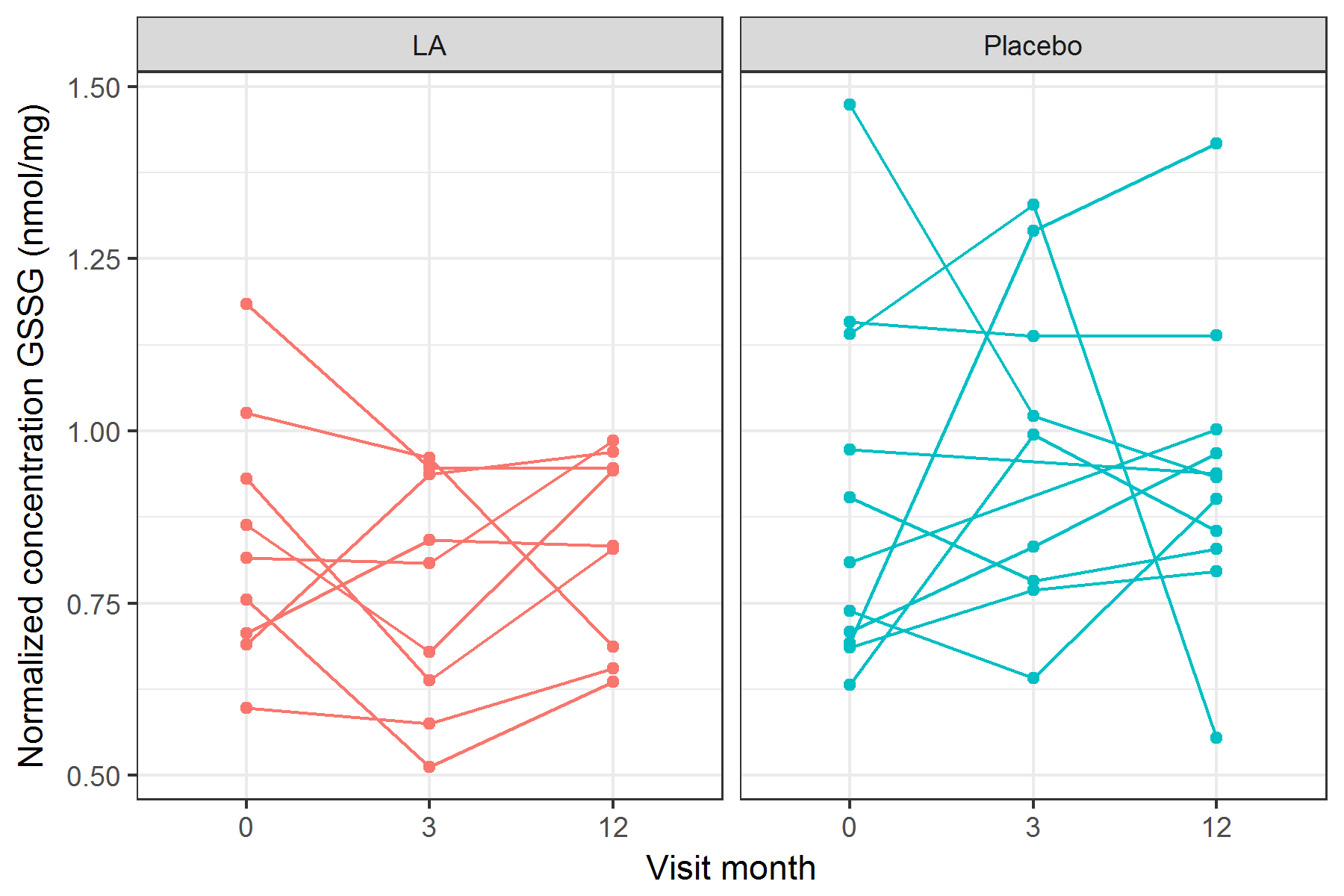
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | df | pvalue |
| (Intercept) | 129.52 | 9.15 | 14.15 | 34 | 0.0000 |
| studyArmPlacebo | -9.39 | 12.34 | -0.76 | 18 | 0.4565 |
| visitMonth3 | -9.74 | 10.99 | -0.89 | 34 | 0.3818 |
| visitMonth12 | 9.21 | 10.99 | 0.84 | 34 | 0.4078 |
| studyArmPlacebo:visitMonth3 | 20.22 | 15.27 | 1.32 | 34 | 0.1942 |
| studyArmPlacebo:visitMonth12 | -1.43 | 14.82 | -0.10 | 34 | 0.9235 |

Details (not shown).

Mixed effects model using the **nlme** package.

*Not executed.* The lme4::lmer() function is good enough.

## Normalized concentration GSSG



figures/lineplotNormalizedConcGSSG.png

Mixed effects model using the **lme4** package.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | df | pvalue |
| (Intercept) | 0.84 | 0.07 | 12.07 | 34 | 0.0000 |
| studyArmPlacebo | 0.06 | 0.09 | 0.64 | 18 | 0.5288 |
| visitMonth3 | -0.07 | 0.09 | -0.85 | 34 | 0.4033 |
| visitMonth12 | -0.01 | 0.09 | -0.11 | 34 | 0.9137 |
| studyArmPlacebo:visitMonth3 | 0.15 | 0.12 | 1.24 | 34 | 0.2232 |
| studyArmPlacebo:visitMonth12 | 0.05 | 0.12 | 0.40 | 34 | 0.6915 |

Details (not shown).

Mixed effects model using the **nlme** package.

*Not executed.* The lme4::lmer() function is good enough.

## Interpretation

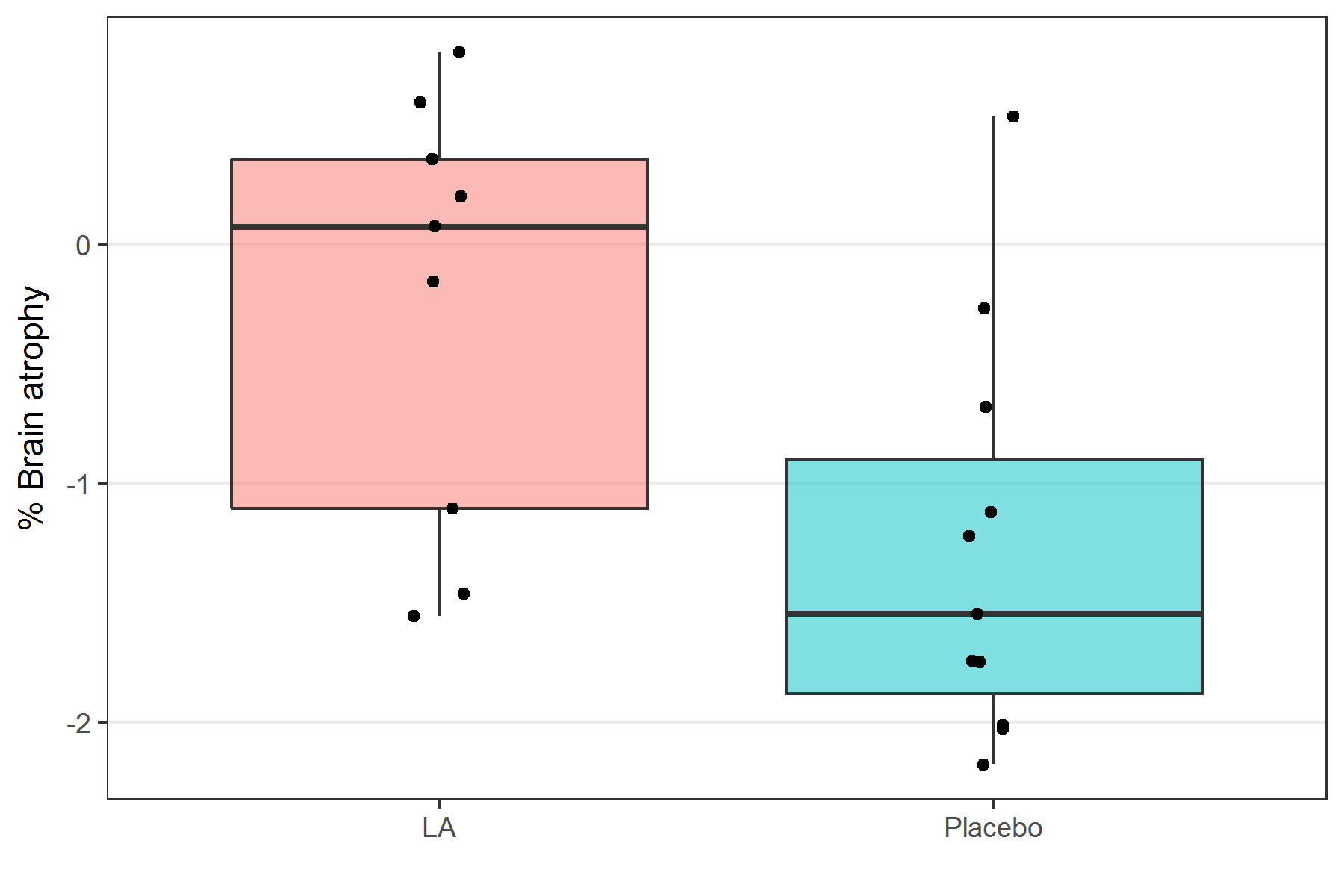
* Normalized concentration ratio was significantly different between placebo and LA
  + Difference between placebo and LA at baseline visit was -20.4 (p-value = 0.0023)
  + Difference between placebo and LA at 3-month visit was -23.5 (p-value = 0.0011)
  + Difference between placebo and LA at 12-month visit was -29.9 (p-value = 6.1 × 10-5)
  + Difference between placebo and LA at baseline visit was not significantly different compared to month 3 or month 12 visits (i.e., difference between placebo and LA was significant at **all visits**)
  + Global difference between placebo and LA was -24.5 (p-value = 1.3 × 10-6)
* Normalized concentration ratio was not significantly different between visits, either within the placebo group or LA group
  + Among LA: difference between 3-month visit and baseline was 2.29 (p-value = 0.7)
  + Among LA: difference between 12-month visit and baseline was 12 (p-value = 0.051)
  + Among placebo: difference between 3-month visit and baseline was -0.769 (p-value = 0.89)
  + Among placebo: difference between 12-month visit and baseline was 2.6 (p-value = 0.63)
* Normalized GSH concentration was not significantly different between study arm or visits
* Normalized GSSG concentration was not significantly different between study arm or visits

# Aim 2

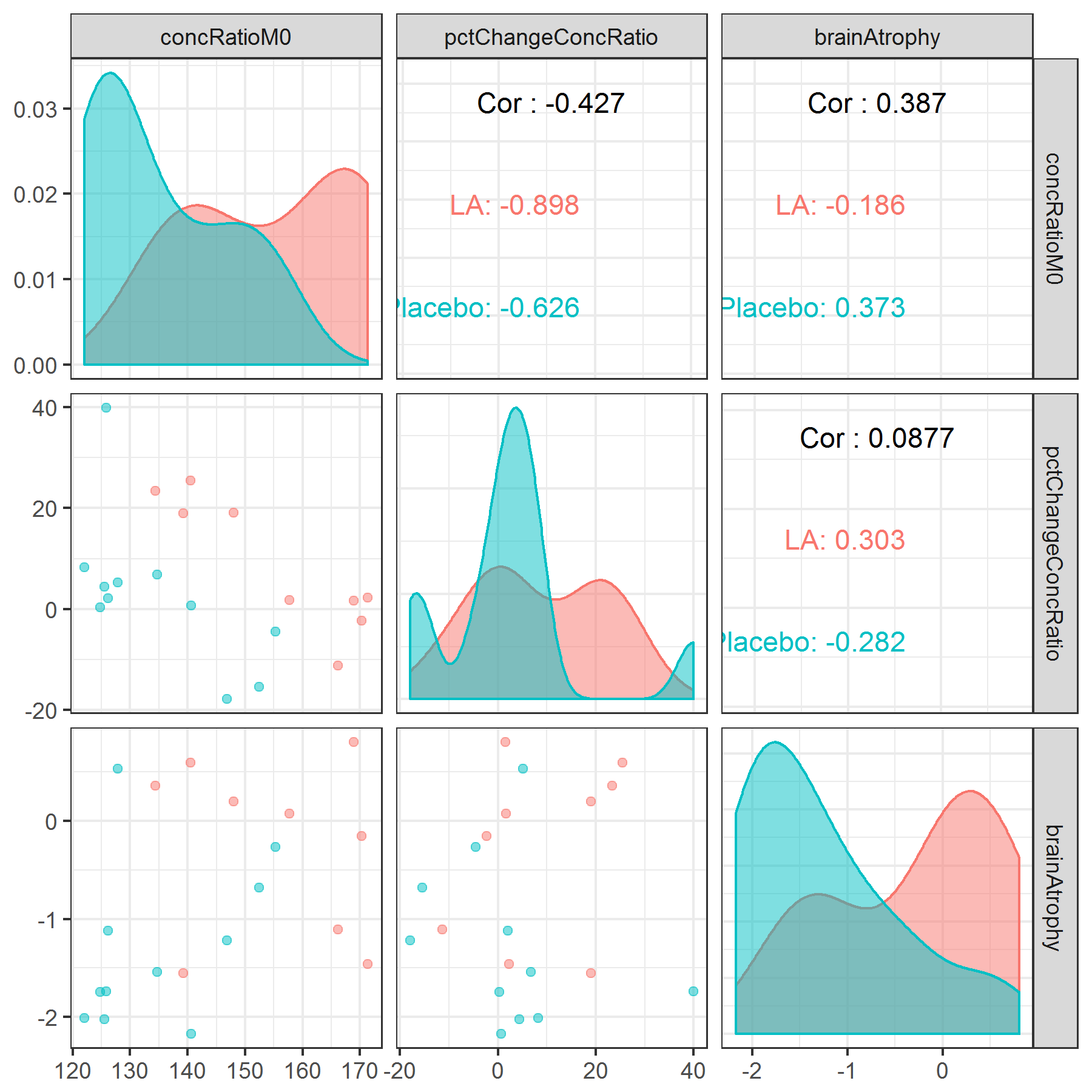
The model for Aim 2 will be a linear regression model.

The model is

## 24-month brain atrophy



figures/boxplotBrainAtrophy.png



figures/scatterplotMatrixBrainAtrophy.png

Linear model.

##   
## Call:  
## lm(formula = brainAtrophy ~ studyArm, data = df2)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -1.30438 -0.74248 0.07256 0.59716 1.80694   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -0.2496 0.2889 -0.864 0.3990   
## studyArmPlacebo -1.0231 0.3895 -2.627 0.0171 \*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.8666 on 18 degrees of freedom  
## Multiple R-squared: 0.2771, Adjusted R-squared: 0.2369   
## F-statistic: 6.899 on 1 and 18 DF, p-value: 0.01711

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | p.value |
| (Intercept) | -0.25 | 0.29 | -0.86 | 0.3990 |
| studyArmPlacebo | -1.02 | 0.39 | -2.63 | 0.0171 |

Details (not shown).

##   
## Call:  
## lm(formula = brainAtrophy ~ studyArm + pctChangeConcRatio, data = df2)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -1.28391 -0.73332 0.04088 0.57735 1.81193   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -0.232099 0.324069 -0.716 0.4836   
## studyArmPlacebo -1.035179 0.410478 -2.522 0.0219 \*  
## pctChangeConcRatio -0.001996 0.014784 -0.135 0.8942   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.8913 on 17 degrees of freedom  
## Multiple R-squared: 0.2779, Adjusted R-squared: 0.1929   
## F-statistic: 3.27 on 2 and 17 DF, p-value: 0.06285

##   
## Call:  
## lm(formula = brainAtrophy ~ studyArm + concRatioM0, data = df2)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -1.30458 -0.67572 -0.00435 0.55146 1.84576   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -1.127281 2.448976 -0.460 0.651   
## studyArmPlacebo -0.907550 0.511691 -1.774 0.094 .  
## concRatioM0 0.005656 0.015666 0.361 0.723   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.8883 on 17 degrees of freedom  
## Multiple R-squared: 0.2826, Adjusted R-squared: 0.1982   
## F-statistic: 3.348 on 2 and 17 DF, p-value: 0.05944

## Interpretation

* Percent brain atrophy was significantly different in the placebo group compared to the LA group
  + Difference of -1.02 (p-value = 0.0171)
* The difference persisted after adjusting for percent change in GSH:GSSG concentration ratio from baseline to 12-month visit
  + Difference of -1.04 (p-value = 0.0219)
* The difference was attenuated after adjusting for baseline GSH:GSSG concentration ratio
  + Difference of -0.908 (p-value = 0.094)