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

Benjamin Chan (chanb@ohsu.edu)

2018-08-14

# 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

From: Carin Waslo  
Sent: Wednesday, June 13, 2018 10:27 AM  
To: Benjamin Chan [chanb@ohsu.edu](mailto:chanb@ohsu.edu)  
Cc: Rebecca Spain [spainr@ohsu.edu](mailto:spainr@ohsu.edu); Cassidy Taylor [taylocas@ohsu.edu](mailto:taylocas@ohsu.edu)  
Subject: SECURE: Spain - Glutathione ratios as the mechanism of action of LA in PMS

Good morning,

I work for Dr. Rebecca Spain and have been given the opportunity to develop a poster for the Glutathione ratios as the mechanism of action of lipoic acid in PMS study. While looking through the results, I noted an error in study arm assignment for PatientID #123. PatientID #123 is listed as having received Placebo, but actually had received LA.

Could you please assign PatientID #123 to the LA study arm and rerun the analysis? This should make each study arm contain 10 subjects. I am sorry for any inconvenience.

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 |
| 123 | 0 | LA | 96.34 | 0.63 | 152.45 |
| 123 | 3 | LA | 131.28 | 1.00 | 131.92 |
| 123 | 12 | LA | 110.30 | 0.86 | 128.95 |
| 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 |
| 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 8 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 1 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 ...  
## $ wholeBrainVol : num 1389025 1437526 1385963 1393046 1413702 ...  
## $ wholeBrainVolScaled: num [1:20, 1] -0.652 0.142 -0.702 -0.586 -0.248 ...  
## ..- attr(\*, "scaled:center")= num 1428831  
## ..- attr(\*, "scaled:scale")= num 61028

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| patientID | studyArm | concRatioM0 | concRatioM12 | pctChangeConcRatio | brainAtrophy | wholeBrainVol | wholeBrainVolScaled |
| 118 | LA | 134.39 | 165.82 | 23.39 | 0.36 | 1389025 | -0.65225435 |
| 123 | LA | 152.45 | 128.95 | -15.41 | -0.68 | 1413702 | -0.24790021 |
| 124 | LA | 168.90 | 171.58 | 1.59 | 0.80 | 1403817 | -0.40987661 |
| 129 | LA | 171.36 | 175.25 | 2.27 | -1.46 | 1394722 | -0.55890808 |
| 130 | LA | 148.02 | 176.26 | 19.08 | 0.20 | 1465597 | 0.60244636 |
| 132 | LA | 139.21 | 165.67 | 19.01 | -1.55 | 1442353 | 0.22157254 |
| 134 | LA | 140.53 | 176.27 | 25.44 | 0.60 | 1494836 | 1.08157144 |
| 140 | LA | 166.15 | 147.36 | -11.31 | -1.11 | 1432413 | 0.05869458 |
| 149 | LA | 157.75 | 160.44 | 1.71 | 0.07 | 1548241 | 1.95665694 |
| 151 | LA | 170.28 | 166.27 | -2.36 | -0.16 | 1463387 | 0.56623855 |
| 119 | Placebo | 125.56 | 131.09 | 4.40 | -2.03 | 1437526 | 0.14247847 |
| 120 | Placebo | 134.69 | 143.82 | 6.78 | -1.54 | 1385963 | -0.70243336 |
| 122 | Placebo | 127.89 | 134.53 | 5.20 | 0.53 | 1393046 | -0.58637150 |
| 125 | Placebo | 126.19 | 128.79 | 2.06 | -1.12 | 1398976 | -0.48919566 |
| 131 | Placebo | 124.82 | 125.19 | 0.29 | -1.75 | 1583152 | 2.52871095 |
| 135 | Placebo | 155.24 | 148.21 | -4.52 | -0.27 | 1460338 | 0.51628125 |
| 139 | Placebo | 140.65 | 141.52 | 0.62 | -2.17 | 1393658 | -0.57634163 |
| 143 | Placebo | 125.84 | 176.13 | 39.96 | -1.74 | 1339494 | -1.46386607 |
| 145 | Placebo | 122.08 | 132.14 | 8.25 | -2.01 | 1355211 | -1.20633292 |
| 153 | Placebo | 146.84 | 120.52 | -17.93 | -1.22 | 1381158 | -0.78117069 |

# Aim 1

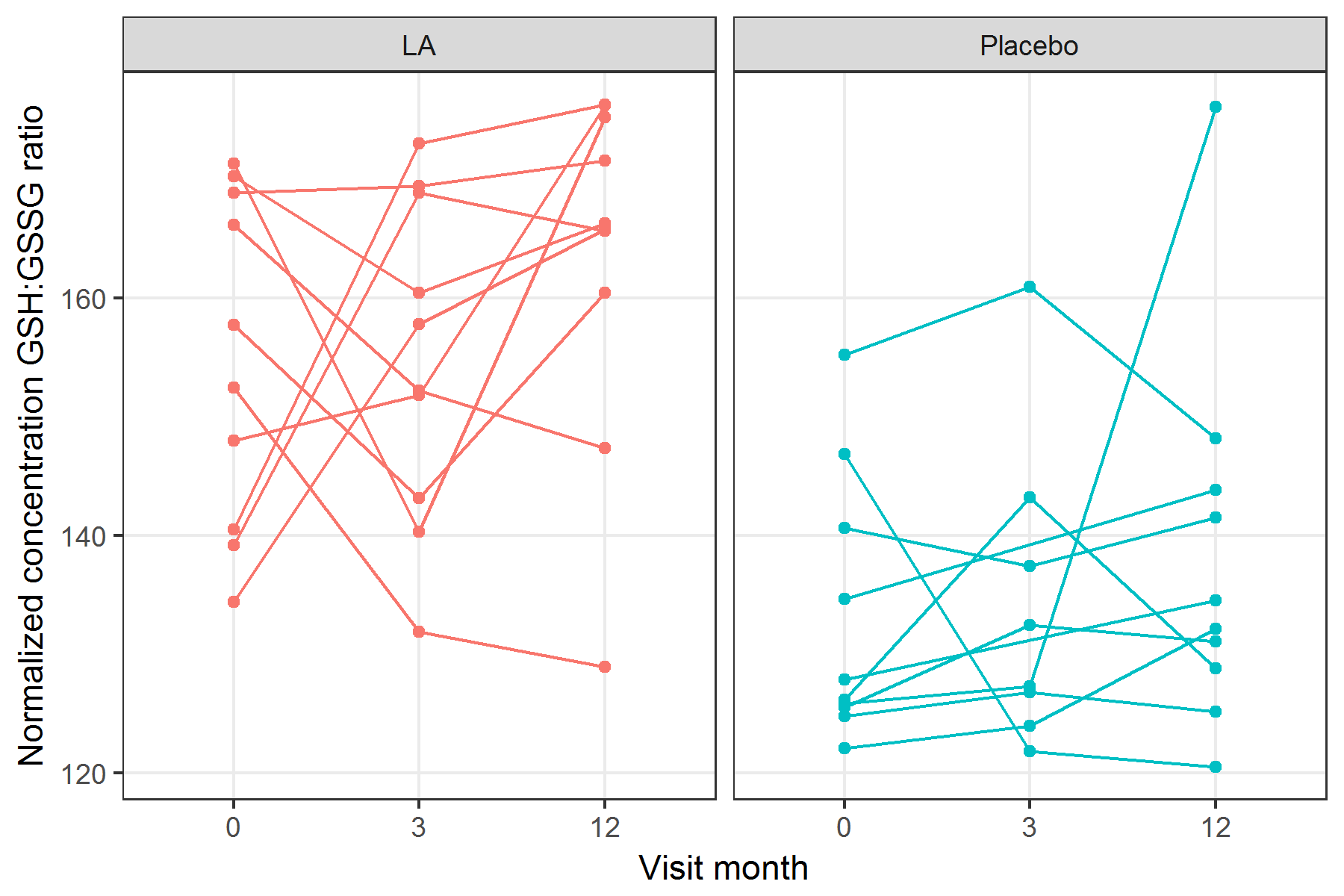
The model for Aim 1 will be a random intercept linear model. Estimation will use the *lme4* package.

##   
## 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},  
## }

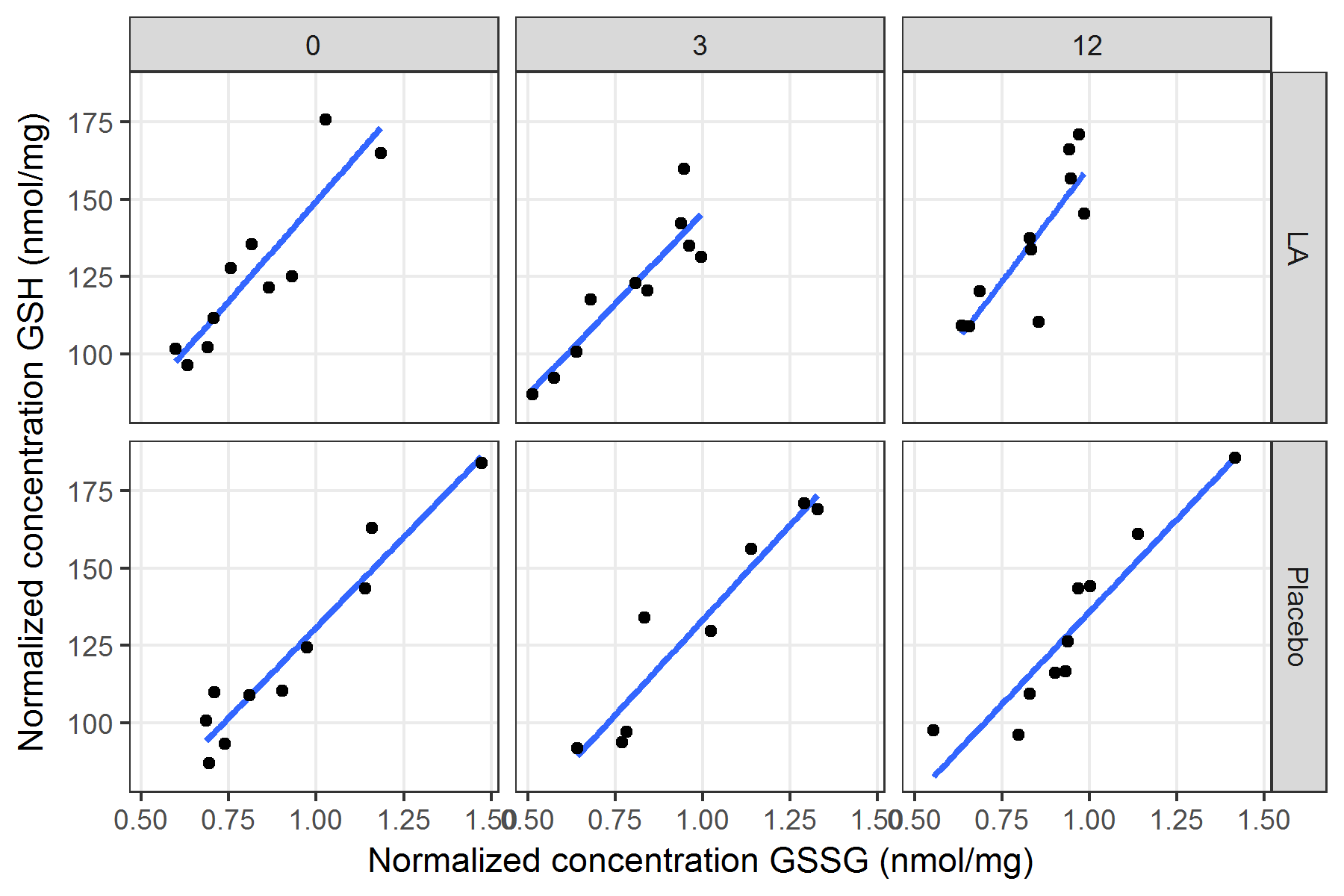
The model is

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) | 154.90 | 4.38 | 35.38 | 34 | 0.0000 |
| studyArmPlacebo | -21.92 | 6.19 | -3.54 | 18 | 0.0023 |
| visitMonth3 | 0.00 | 5.73 | 0.00 | 34 | 0.9995 |
| visitMonth12 | 8.48 | 5.73 | 1.48 | 34 | 0.1478 |
| studyArmPlacebo:visitMonth3 | 1.24 | 8.38 | 0.15 | 34 | 0.8830 |
| studyArmPlacebo:visitMonth12 | -3.27 | 8.10 | -0.40 | 34 | 0.6891 |

## Note: uncertainty of the random effects parameters are not taken into account for confidence intervals.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| group | x | predicted | conf.low | conf.high |
| LA | 0 | 154.9033 | 146.3226 | 163.4839 |
| LA | 3 | 154.9072 | 146.3266 | 163.4879 |
| LA | 12 | 163.3880 | 154.8073 | 171.9686 |
| Placebo | 0 | 132.9788 | 124.3982 | 141.5595 |
| Placebo | 3 | 134.2257 | 125.6450 | 142.8063 |
| Placebo | 12 | 138.1935 | 129.6128 | 146.7741 |

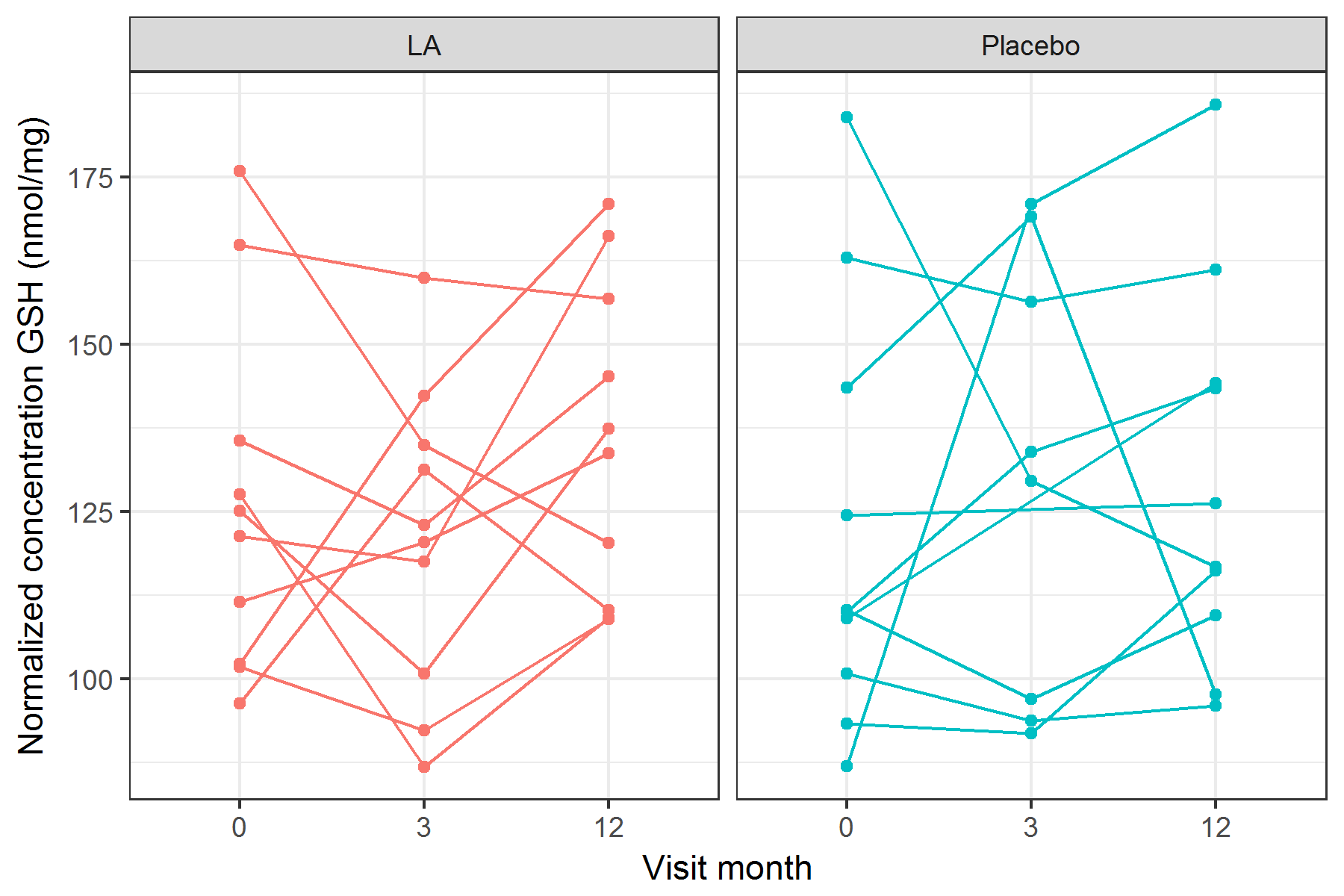
## Note: uncertainty of the random effects parameters are not taken into account for confidence intervals.

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.

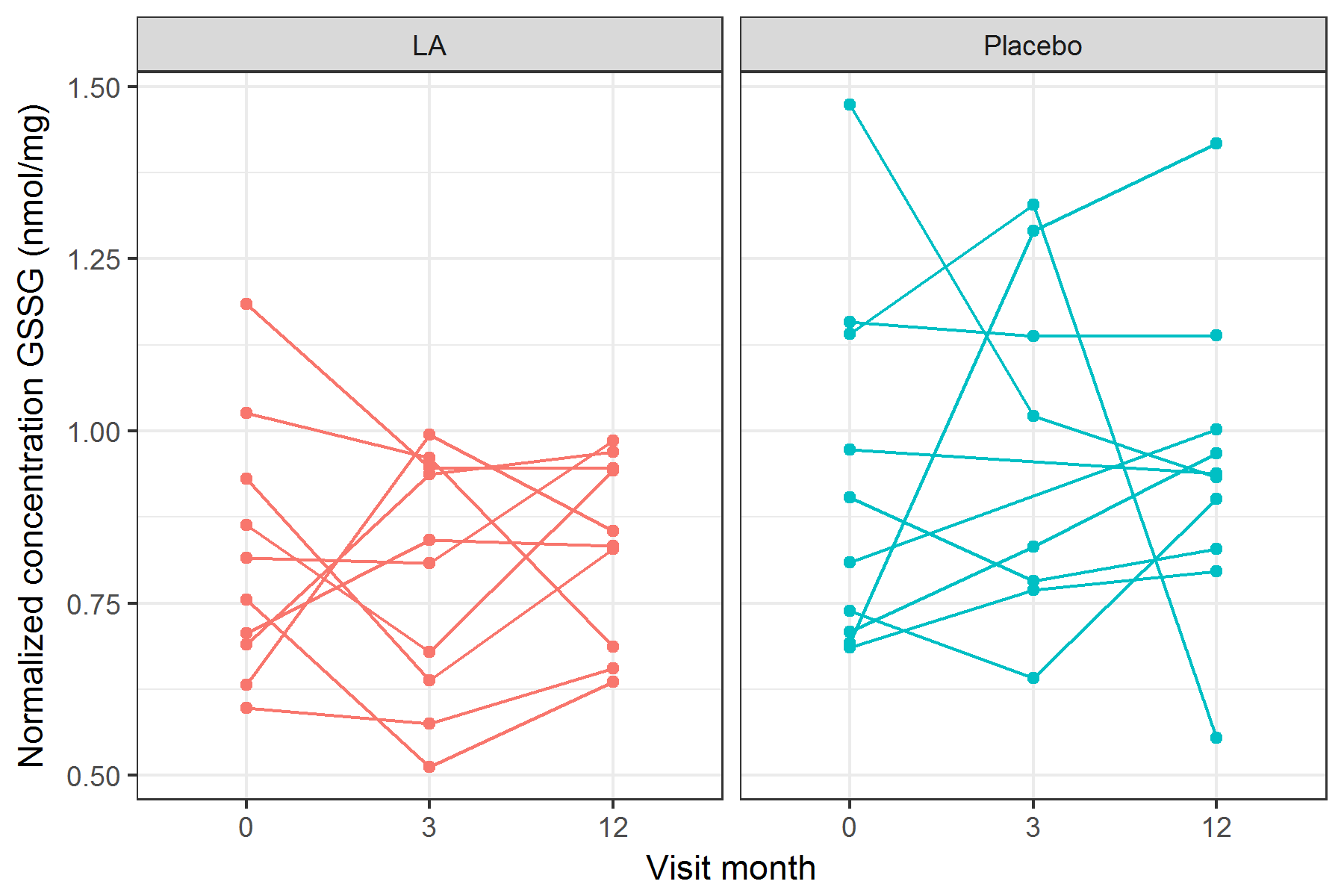
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | df | pvalue |
| (Intercept) | 126.20 | 8.78 | 14.38 | 34 | 0.0000 |
| studyArmPlacebo | -3.69 | 12.41 | -0.30 | 18 | 0.7694 |
| visitMonth3 | -5.27 | 10.63 | -0.50 | 34 | 0.6230 |
| visitMonth12 | 9.69 | 10.63 | 0.91 | 34 | 0.3685 |
| studyArmPlacebo:visitMonth3 | 13.07 | 15.59 | 0.84 | 34 | 0.4077 |
| studyArmPlacebo:visitMonth12 | -2.52 | 15.03 | -0.17 | 34 | 0.8676 |

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) | 0.82 | 0.07 | 12.41 | 34 | 0.0000 |
| studyArmPlacebo | 0.11 | 0.09 | 1.16 | 18 | 0.2621 |
| visitMonth3 | -0.03 | 0.08 | -0.36 | 34 | 0.7196 |
| visitMonth12 | 0.01 | 0.08 | 0.16 | 34 | 0.8726 |
| studyArmPlacebo:visitMonth3 | 0.08 | 0.12 | 0.62 | 34 | 0.5383 |
| studyArmPlacebo:visitMonth12 | 0.01 | 0.12 | 0.05 | 34 | 0.9629 |

Details (not shown).

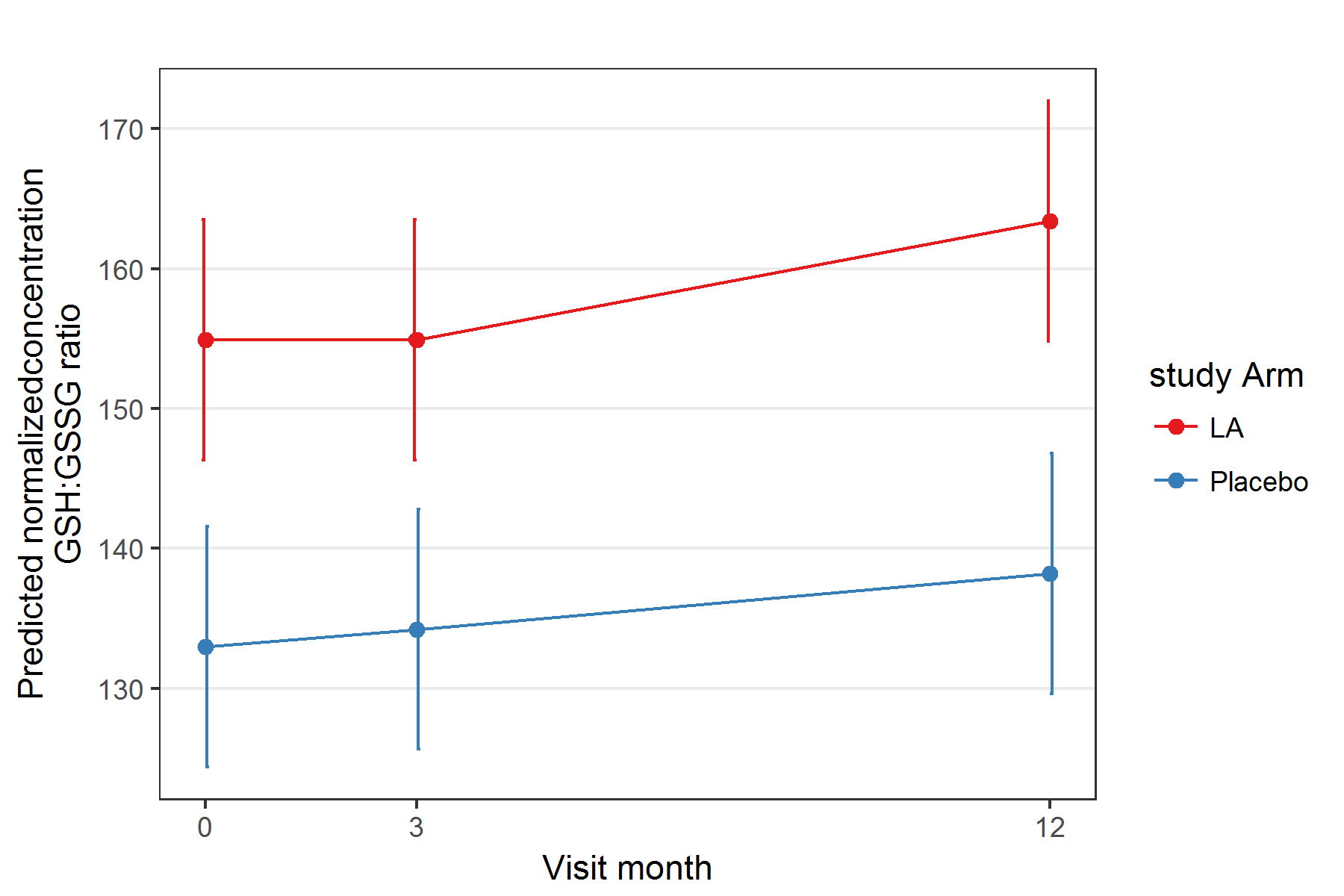
Mixed effects model using the **nlme** package.

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

## Interpretation

### Normalized concentration ratio

* Normalized concentration ratio was significantly different between placebo and LA
  + Difference between placebo and LA at baseline visit was -21.9 (p-value = 0.0023)
  + Difference between placebo and LA at 3-month visit was -20.7 (p-value = 0.0055)
  + Difference between placebo and LA at 12-month visit was -25.2 (p-value = 7.2 × 10-4)
  + 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 -22.5 (p-value = 3.3 × 10-5)
* 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 0.00397 (p-value = 1)
  + Among LA: difference between 12-month visit and baseline was 8.48 (p-value = 0.15)
  + Among placebo: difference between 3-month visit and baseline was 1.25 (p-value = 0.84)
  + Among placebo: difference between 12-month visit and baseline was 5.21 (p-value = 0.37)
* The differences in normalized concentration ratio from baseline were not significantly different between LA and placebo
  + Baseline to 3-months: 1.24 (p-value = 0.88)
  + Baseline to 12-months: -3.27 (p-value = 0.69)



figures/predictedNormalizedConcRatio.png

### Normalized GSH concentration

* Normalized GSH concentration was not significantly different between study arm or visits

### Normalized GSSG concentration

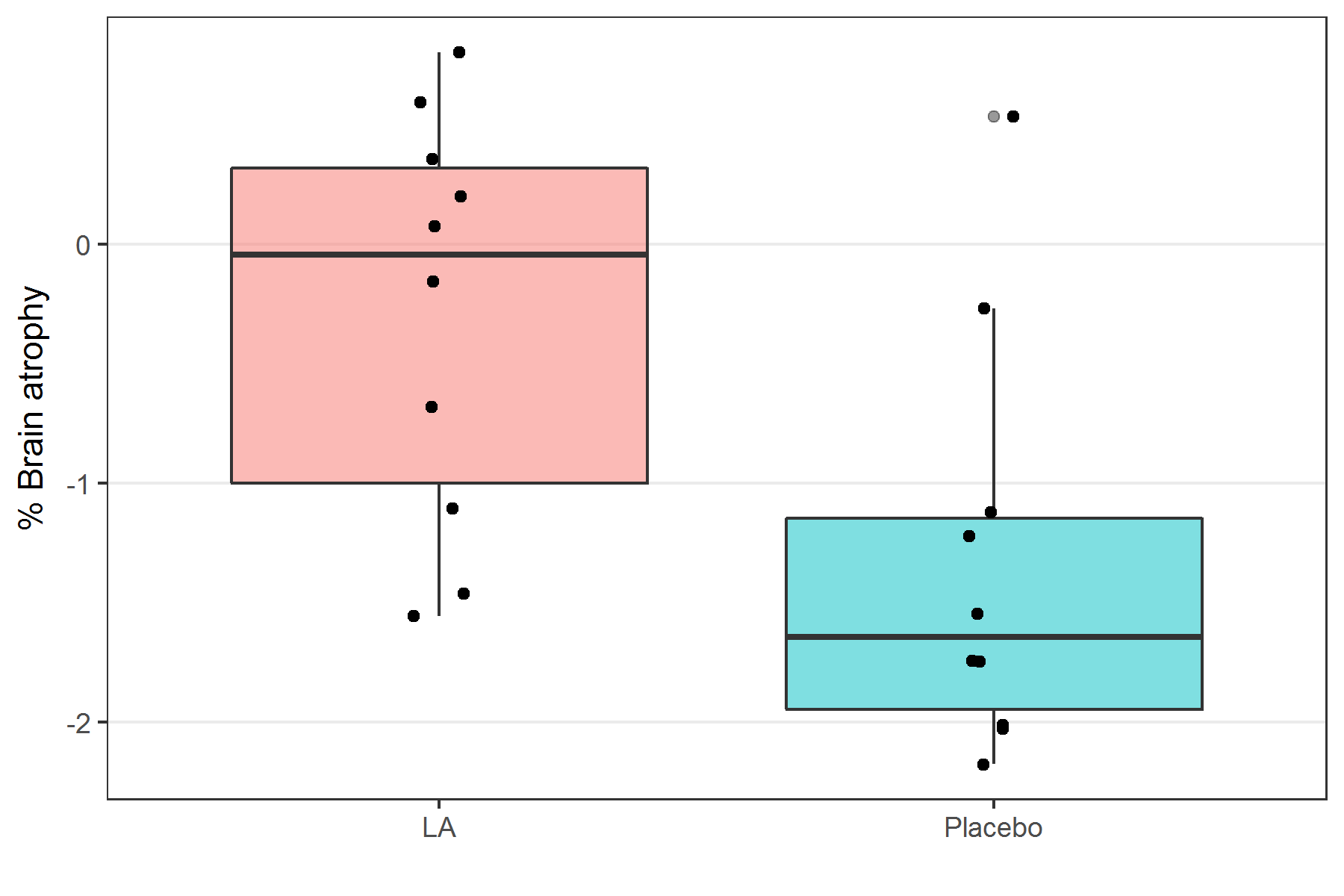
* Normalized GSSG concentration was not significantly different between study arm or visits

# Aim 2

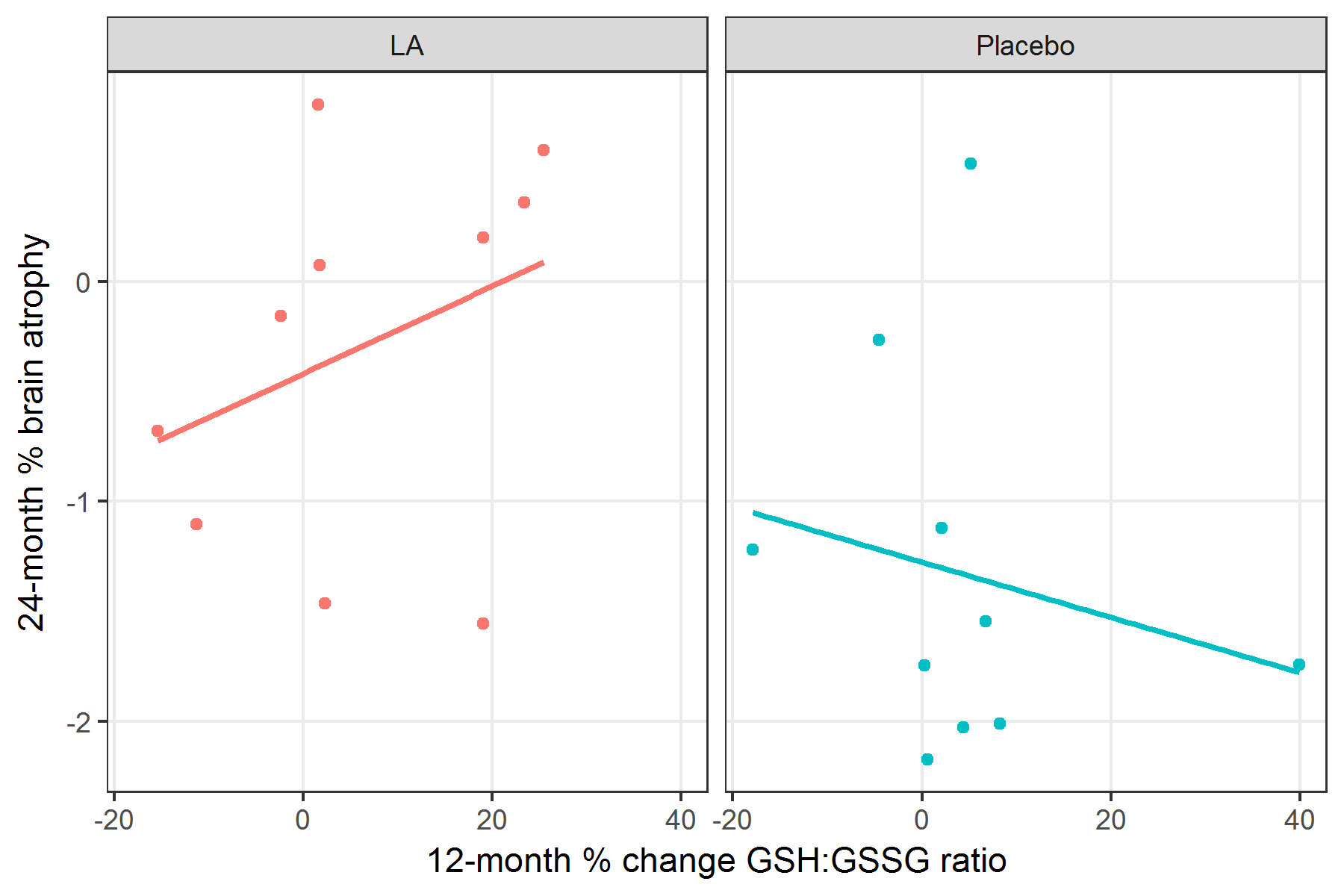
The model for Aim 2 will be a linear regression model.

The model is

## 24-month brain atrophy



figures/boxplotBrainAtrophy.png



figures/scatterplotBrainAtrophy.png

Linear model.

##   
## Call:  
## lm(formula = brainAtrophy ~ pctChangeConcRatio, data = df2)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -1.3330 -0.8445 -0.2278 0.9139 1.6401   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -0.845579 0.243916 -3.467 0.00275 \*\*  
## pctChangeConcRatio 0.006138 0.016436 0.373 0.71316   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 1.015 on 18 degrees of freedom  
## Multiple R-squared: 0.007689, Adjusted R-squared: -0.04744   
## F-statistic: 0.1395 on 1 and 18 DF, p-value: 0.7132

##   
## Call:  
## lm(formula = brainAtrophy ~ pctChangeConcRatio + studyArm, data = df2)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -1.30784 -0.69327 -0.02638 0.48161 1.86379   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -0.315742 0.293528 -1.076 0.2971   
## pctChangeConcRatio 0.003663 0.014321 0.256 0.8012   
## studyArmPlacebo -1.032810 0.395621 -2.611 0.0183 \*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.8827 on 17 degrees of freedom  
## Multiple R-squared: 0.2917, Adjusted R-squared: 0.2083   
## F-statistic: 3.5 on 2 and 17 DF, p-value: 0.05334

##   
## Call:  
## lm(formula = brainAtrophy ~ pctChangeConcRatio + studyArm + pctChangeConcRatio:studyArm,   
## data = df2)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -1.51347 -0.50883 0.03996 0.34823 1.87493   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -0.41864 0.30452 -1.375 0.1882   
## pctChangeConcRatio 0.01989 0.02008 0.991 0.3365   
## studyArmPlacebo -0.85676 0.42126 -2.034 0.0589 .  
## pctChangeConcRatio:studyArmPlacebo -0.03245 0.02839 -1.143 0.2698   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.8748 on 16 degrees of freedom  
## Multiple R-squared: 0.3451, Adjusted R-squared: 0.2224   
## F-statistic: 2.811 on 3 and 16 DF, p-value: 0.07279

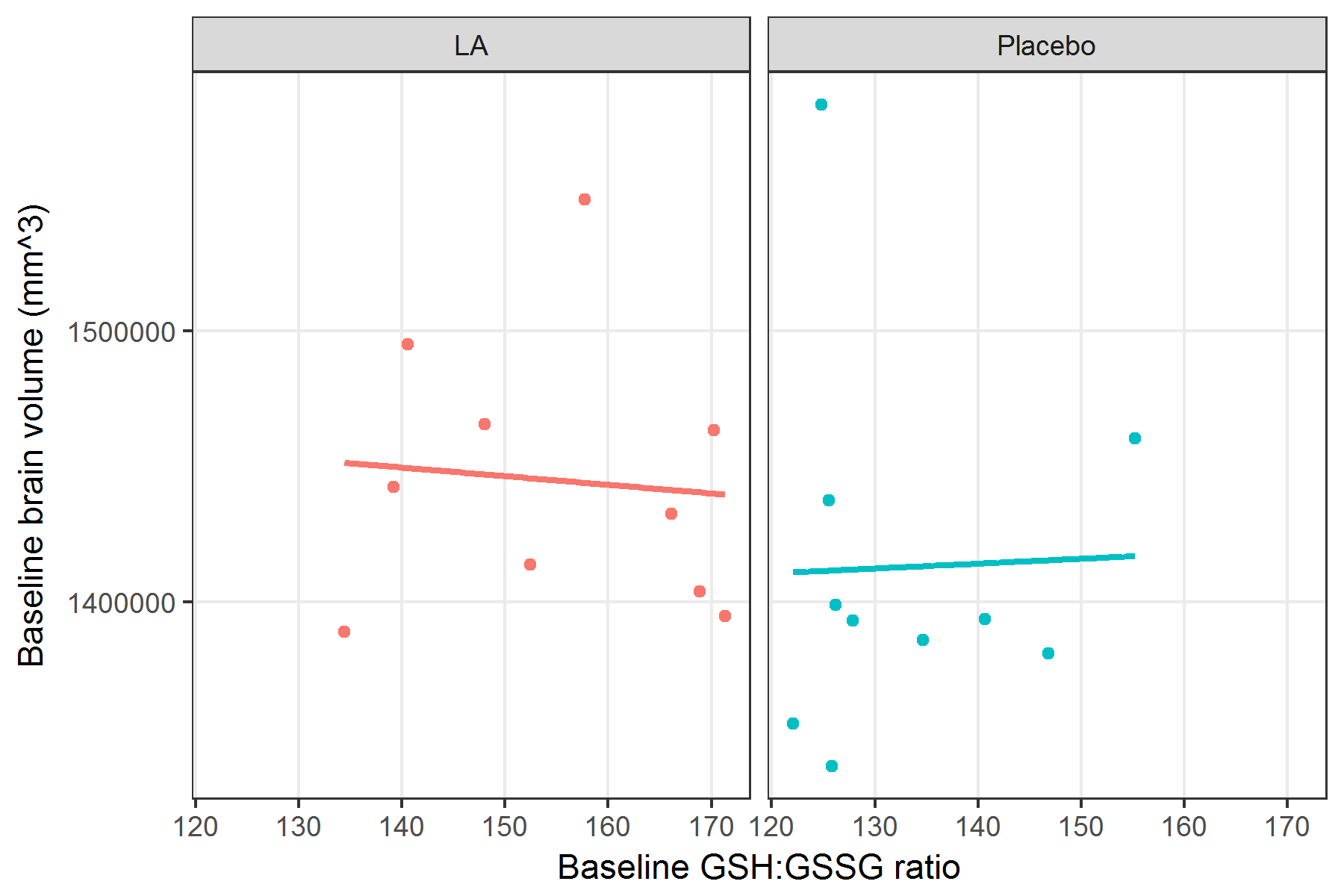
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | p.value |
| (Intercept) | -0.42 | 0.30 | -1.37 | 0.1882 |
| pctChangeConcRatio | 0.02 | 0.02 | 0.99 | 0.3365 |
| studyArmPlacebo | -0.86 | 0.42 | -2.03 | 0.0589 |
| pctChangeConcRatio:studyArmPlacebo | -0.03 | 0.03 | -1.14 | 0.2698 |

Details (not shown).

## Interpretation

* Percent brain atrophy was not significantly associated with GSH:GSSG concentration change
  + of 0.00614 (p-value = 0.713)
  + Correlation coefficient = 0.0877
  + GSH:GSSG concentration change explains 0.769% of the variation in brain atrophy
* The association was still not significant after adjusting for study arm
  + of 0.00366 (p-value = 0.801)
  + Partial correlation coefficient = 0.0619
  + GSH:GSSG concentration change explains 0.383% of the variation in brain atrophy after adjusting for study arm

## Crosssectional correlation between baseline GSH:GSSG ratio and brain volume



figures/scatterplotBrainVolumeAtBaseline.png

Linear model.

##   
## Call:  
## lm(formula = wholeBrainVol ~ concRatioM0, data = df2)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -78569 -37987 -19742 20919 165697   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) 1343187.3 123322.1 10.892 2.36e-09 \*\*\*  
## concRatioM0 595.0 851.3 0.699 0.494   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 61870 on 18 degrees of freedom  
## Multiple R-squared: 0.02642, Adjusted R-squared: -0.02767   
## F-statistic: 0.4884 on 1 and 18 DF, p-value: 0.4936

##   
## Call:  
## lm(formula = wholeBrainVol ~ concRatioM0 + studyArm, data = df2)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -74254 -33370 -16480 21317 169276   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) 1464256.0 180476.2 8.113 3.01e-07 \*\*\*  
## concRatioM0 -125.5 1158.2 -0.108 0.915   
## studyArmPlacebo -34709.5 37638.6 -0.922 0.369   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 62120 on 17 degrees of freedom  
## Multiple R-squared: 0.0728, Adjusted R-squared: -0.03628   
## F-statistic: 0.6674 on 2 and 17 DF, p-value: 0.526

### Interpretation

* Baseline whole brain volume was not significantly associated with baseline GSH:GSSG concentration ratio
  + of 595 (p-value = 0.494)
  + Correlation coefficient = 0.163
  + Baseline GSH:GSSG concentration ratio explains 2.64% of the variation in brain atrophy
* The association was still not significant after adjusting for study arm
  + of -126 (p-value = 0.915)
  + Partial correlation coefficient = -0.0263
  + Baseline GSH:GSSG concentration ratio explains 0.0691% of the variation in brain atrophy after adjusting for study arm

# Additional analysis

From: Carin Waslo  
Sent: Wednesday, August 08, 2018 3:27 PM  
To: Benjamin Chan [chanb@ohsu.edu](mailto:chanb@ohsu.edu)  
Subject: Spain - Glutathione ratios as the mechanism of action of LA in PMS

Good afternoon,

Dr. Spain and I are developing a poster and abstract, for the Glutathione study, to submit the ACRIMS 2019. Would you be willing to 1) review the attached abstract and poster to ensure correct interpretation of the results you provided, and 2) run one last analysis to see if baseline GSH:GSSG ratios “predict” subsequent (at month 24) % change brain volumes for LA and placebo groups?

Please let me know if you have any questions or need more information. And thank you very much!

Best, Carin

The model closely follows the model used for Aim 2.

The model is

Linear model.

##   
## Call:  
## lm(formula = brainAtrophy ~ concRatioM0, data = df2)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -1.2866 -0.6484 -0.2690 0.6557 1.7164   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -4.12773 1.87335 -2.203 0.0408 \*  
## concRatioM0 0.02303 0.01293 1.781 0.0918 .  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.9398 on 18 degrees of freedom  
## Multiple R-squared: 0.1498, Adjusted R-squared: 0.1026   
## F-statistic: 3.172 on 1 and 18 DF, p-value: 0.09179

##   
## Call:  
## lm(formula = brainAtrophy ~ concRatioM0 + studyArm, data = df2)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -1.21955 -0.65633 -0.07133 0.55967 1.87990   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -0.705971 2.567220 -0.275 0.7866   
## concRatioM0 0.002669 0.016475 0.162 0.8732   
## studyArmPlacebo -0.980992 0.535398 -1.832 0.0845 .  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.8837 on 17 degrees of freedom  
## Multiple R-squared: 0.29, Adjusted R-squared: 0.2065   
## F-statistic: 3.472 on 2 and 17 DF, p-value: 0.05439

##   
## Call:  
## lm(formula = brainAtrophy ~ concRatioM0 + studyArm + concRatioM0:studyArm,   
## data = df2)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -1.4275 -0.4456 -0.2192 0.4234 1.9869   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)  
## (Intercept) 1.34601 3.26793 0.412 0.686  
## concRatioM0 -0.01058 0.02102 -0.503 0.622  
## studyArmPlacebo -5.82711 4.81142 -1.211 0.243  
## concRatioM0:studyArmPlacebo 0.03426 0.03380 1.013 0.326  
##   
## Residual standard error: 0.883 on 16 degrees of freedom  
## Multiple R-squared: 0.3329, Adjusted R-squared: 0.2078   
## F-statistic: 2.661 on 3 and 16 DF, p-value: 0.08332

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | p.value |
| (Intercept) | 1.35 | 3.27 | 0.41 | 0.6859 |
| concRatioM0 | -0.01 | 0.02 | -0.50 | 0.6217 |
| studyArmPlacebo | -5.83 | 4.81 | -1.21 | 0.2434 |
| concRatioM0:studyArmPlacebo | 0.03 | 0.03 | 1.01 | 0.3259 |

Details (not shown).

## Interpretation

* Percent brain atrophy was not significantly associated with baseline GSH:GSSG concentration
  + of 0.023 (p-value = 0.0918)
  + Correlation coefficient = 0.387
  + Baseline GSH:GSSG concentration explains 15% of the variation in brain atrophy
* The association was still not significant after adjusting for study arm
  + of 0.00267 (p-value = 0.873)
  + Partial correlation coefficient = 0.0393
  + Baseline GSH:GSSG concentration explains 0.154% of the variation in brain atrophy after adjusting for study arm