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 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 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 ...  
## $ 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 |
| 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 |
| 123 | Placebo | 152.45 | 128.95 | -15.41 | -0.68 | 1413702 | -0.24790021 |
| 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

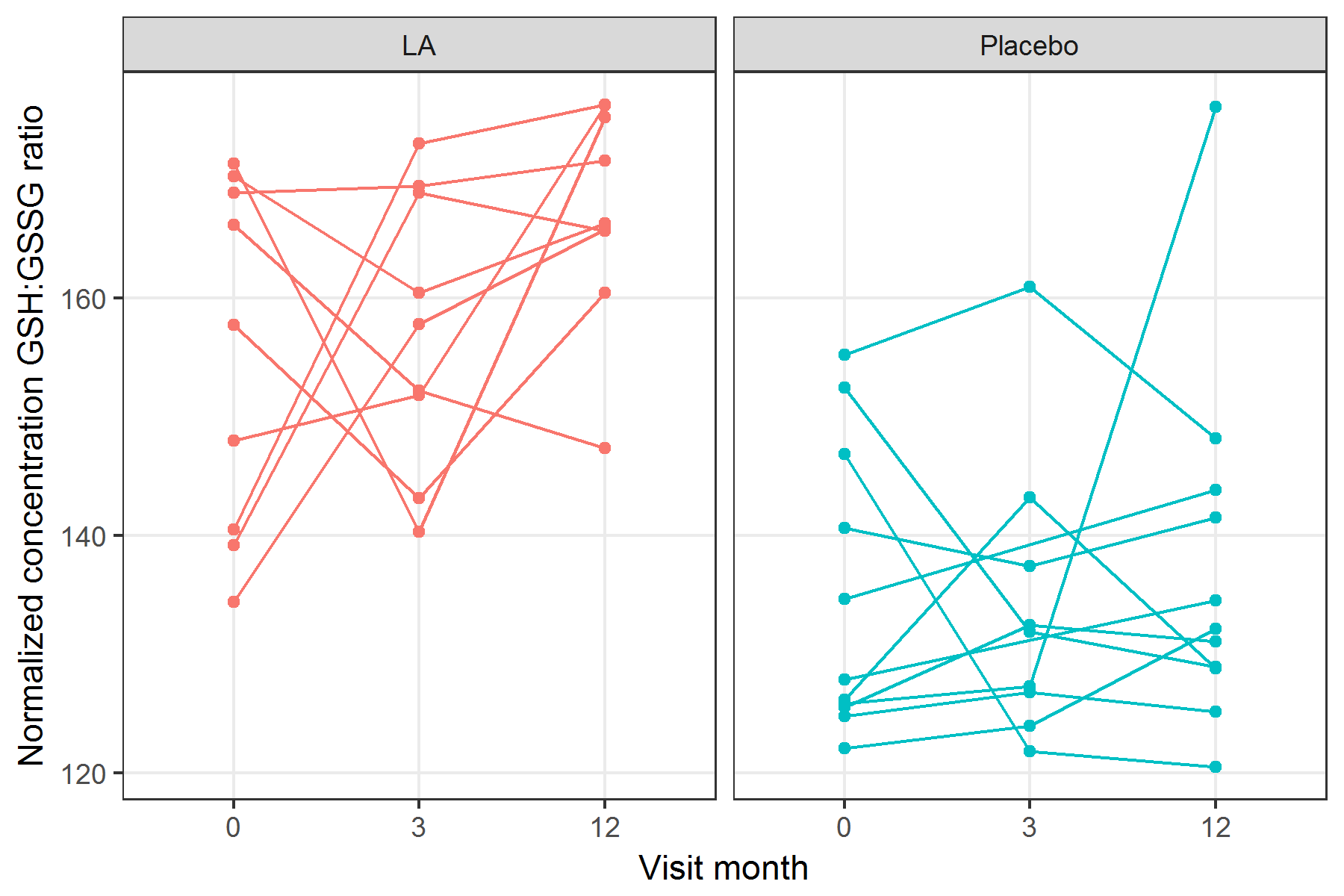
The model for Aim 1 will be a random intercept linear model. Estimation will use the *lme4* package.

##   
## 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},  
## }

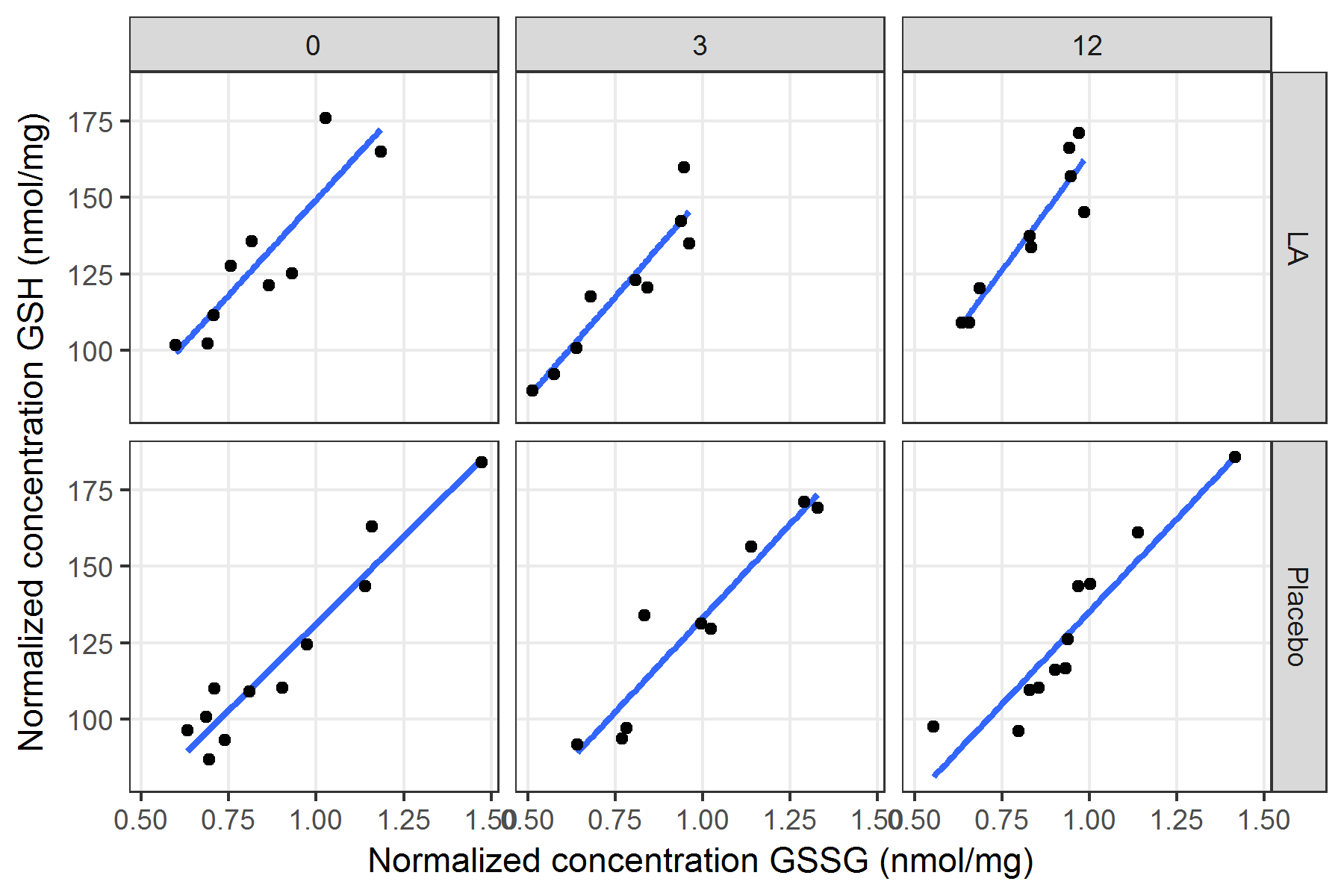
The model is

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) | 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 |

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| group | x | predicted | conf.low | conf.high |
| LA | 0 | 155.1758 | 146.8258 | 163.5257 |
| LA | 3 | 157.4609 | 149.1109 | 165.8108 |
| LA | 12 | 167.2139 | 158.8640 | 175.5639 |
| Placebo | 0 | 134.7490 | 126.3991 | 143.0989 |
| Placebo | 3 | 133.9797 | 125.6297 | 142.3296 |
| Placebo | 12 | 137.3535 | 129.0036 | 145.7035 |

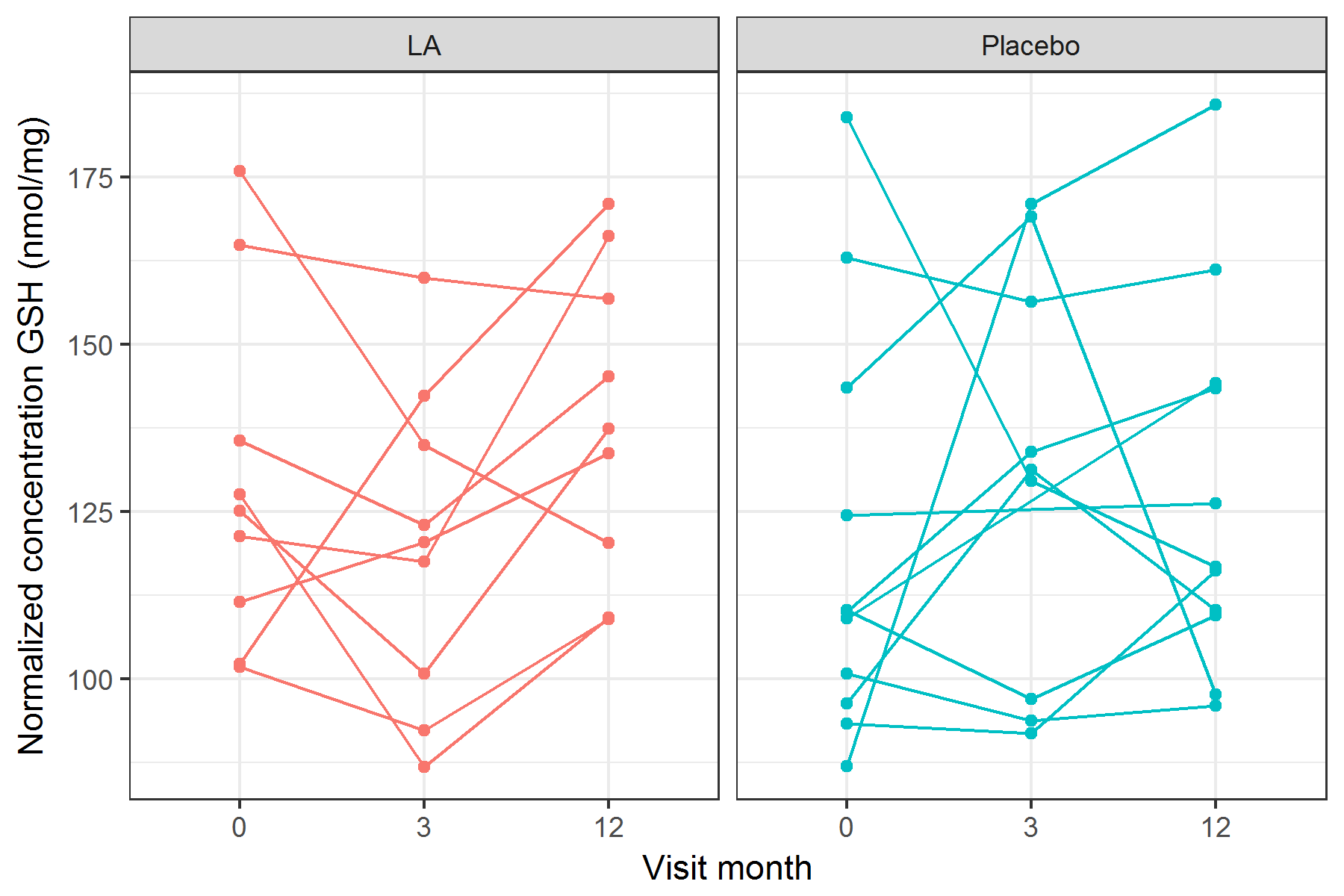
## Note: uncertainty of the random effects parameters are not taken into account for confidence intervals.

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.

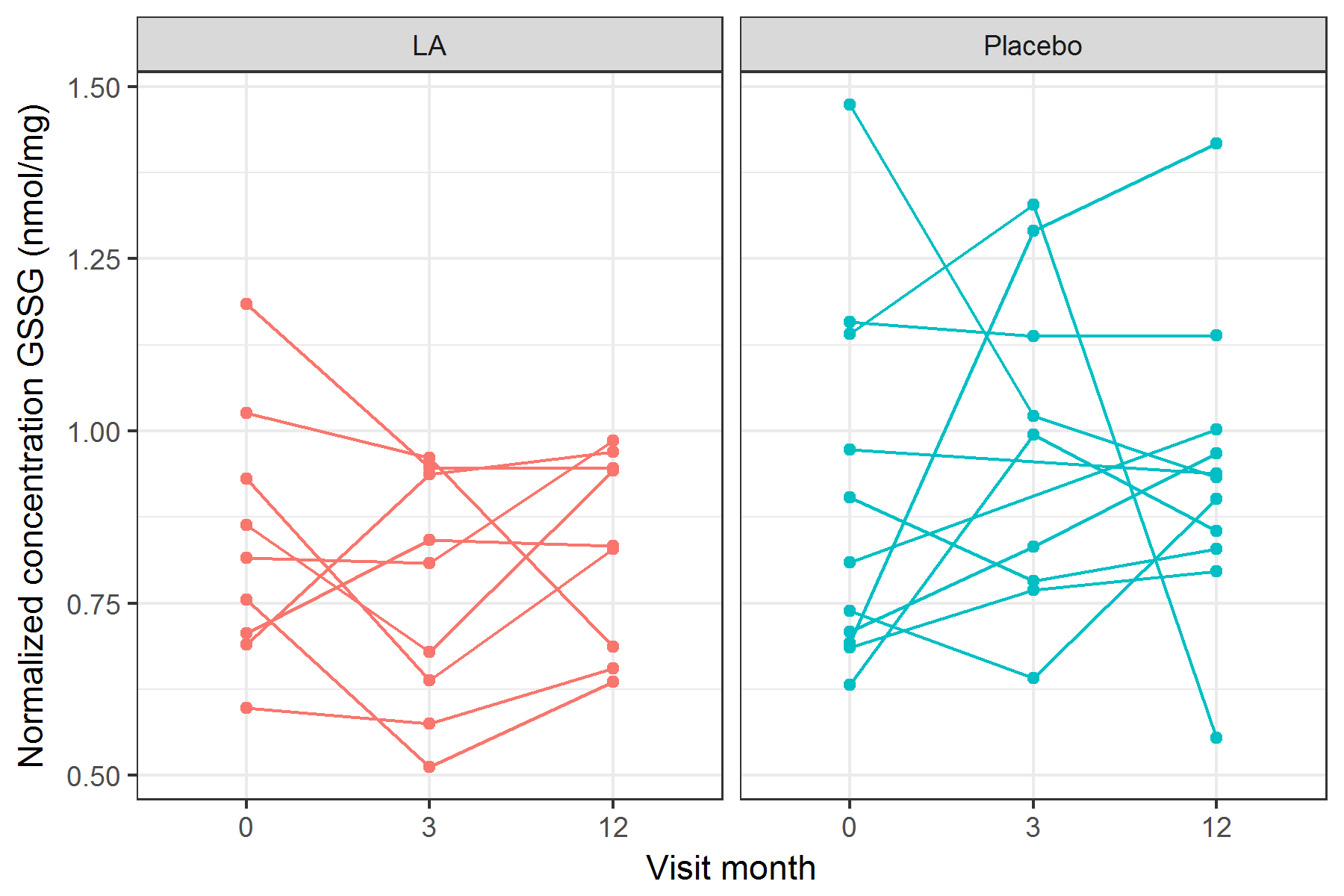
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | df | pvalue |
| (Intercept) | 129.52 | 9.15 | 14.15 | 34 | 0.0000 |
| studyArmPlacebo | -9.39 | 12.34 | -0.76 | 18 | 0.4565 |
| visitMonth3 | -9.74 | 10.99 | -0.89 | 34 | 0.3818 |
| visitMonth12 | 9.21 | 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).

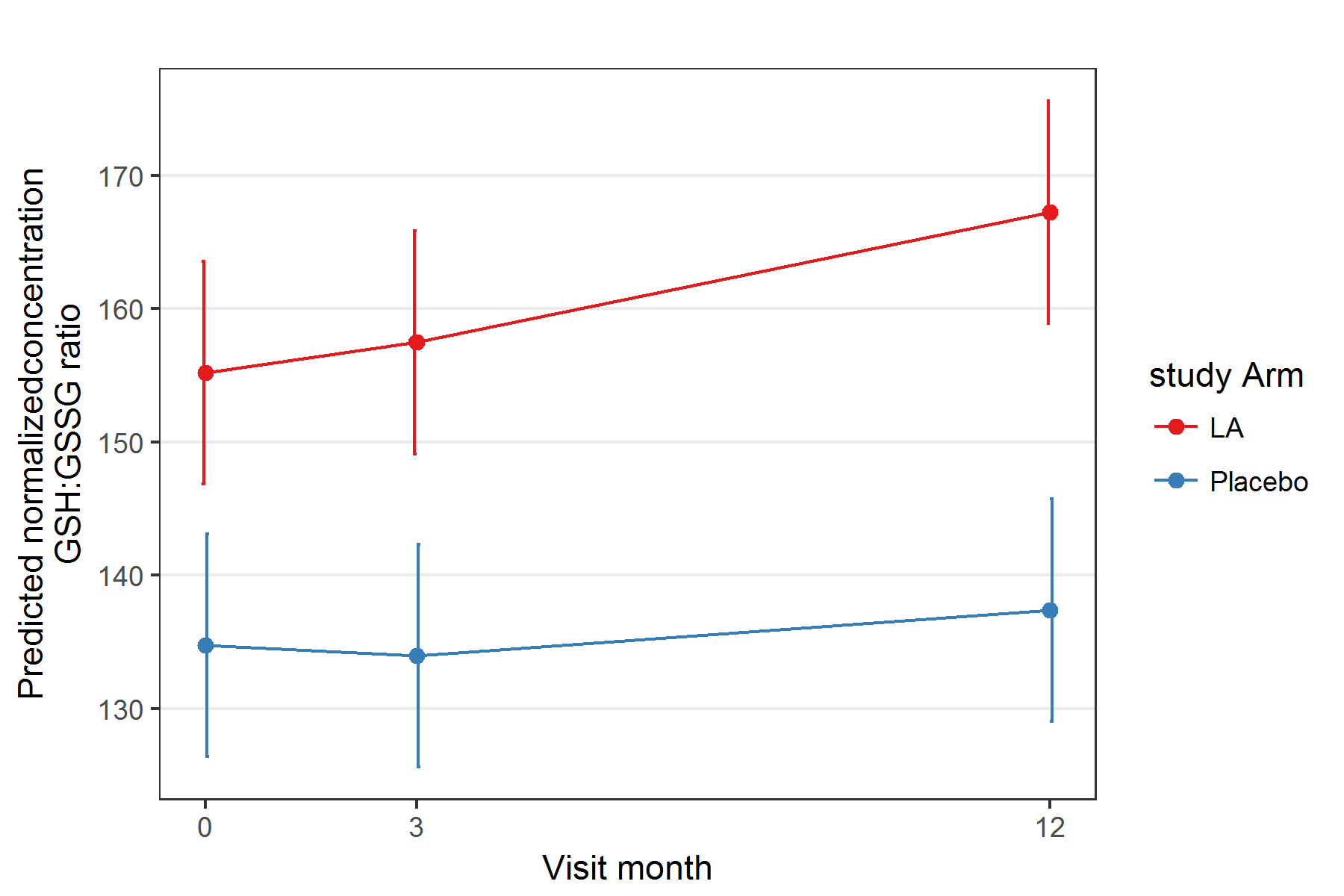
Mixed effects model using the **nlme** package.

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

## Interpretation

### Normalized concentration ratio

* 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)
* The differences in normalized concentration ratio from baseline were not significantly different between LA and placebo
  + Baseline to 3-months: -3.05 (p-value = 0.71)
  + Baseline to 12-months: -9.43 (p-value = 0.25)



figures/predictedNormalizedConcRatio.png

### Normalized GSH concentration

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

### Normalized GSSG concentration

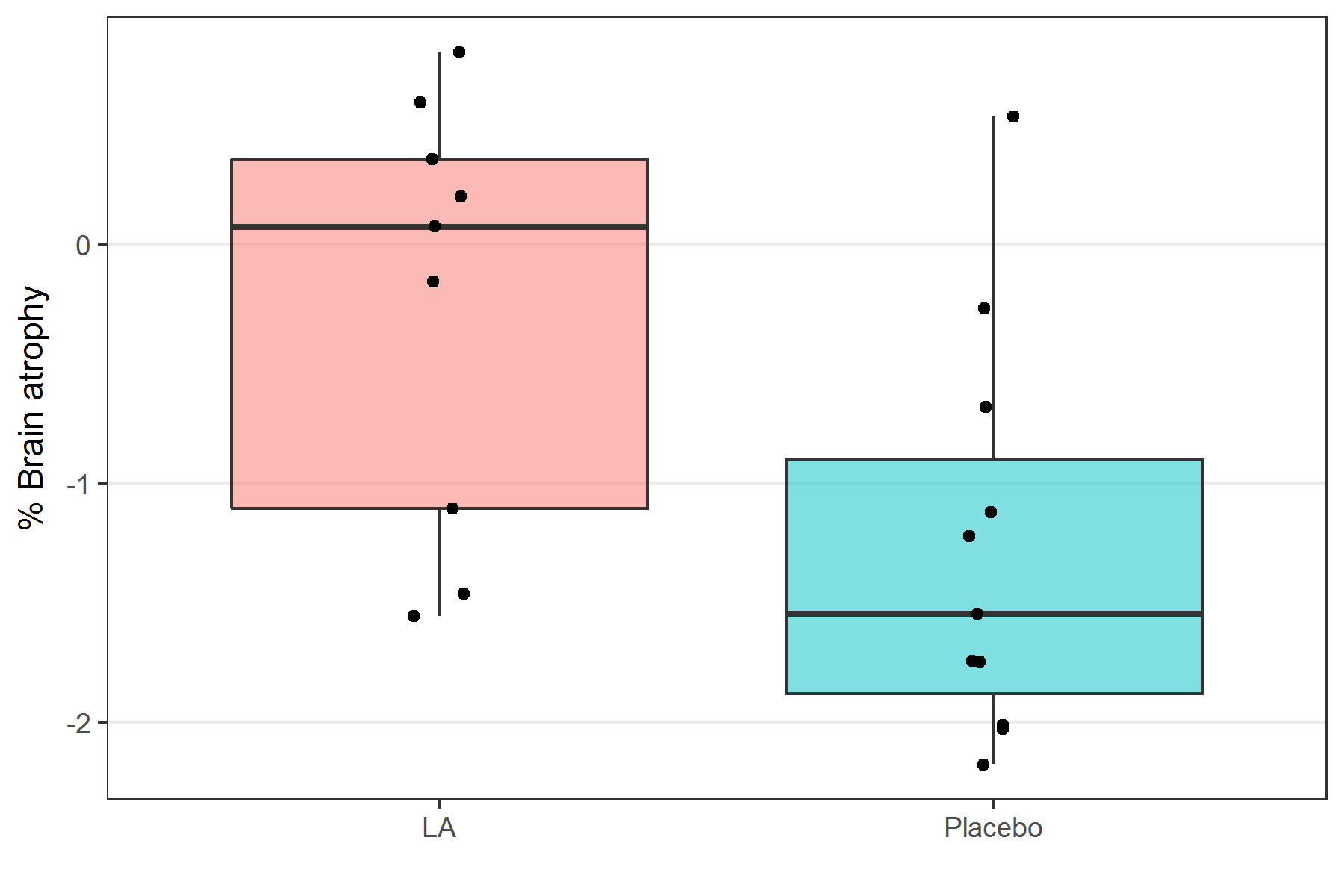
* Normalized GSSG concentration was not significantly different between study arm or visits

# Aim 2

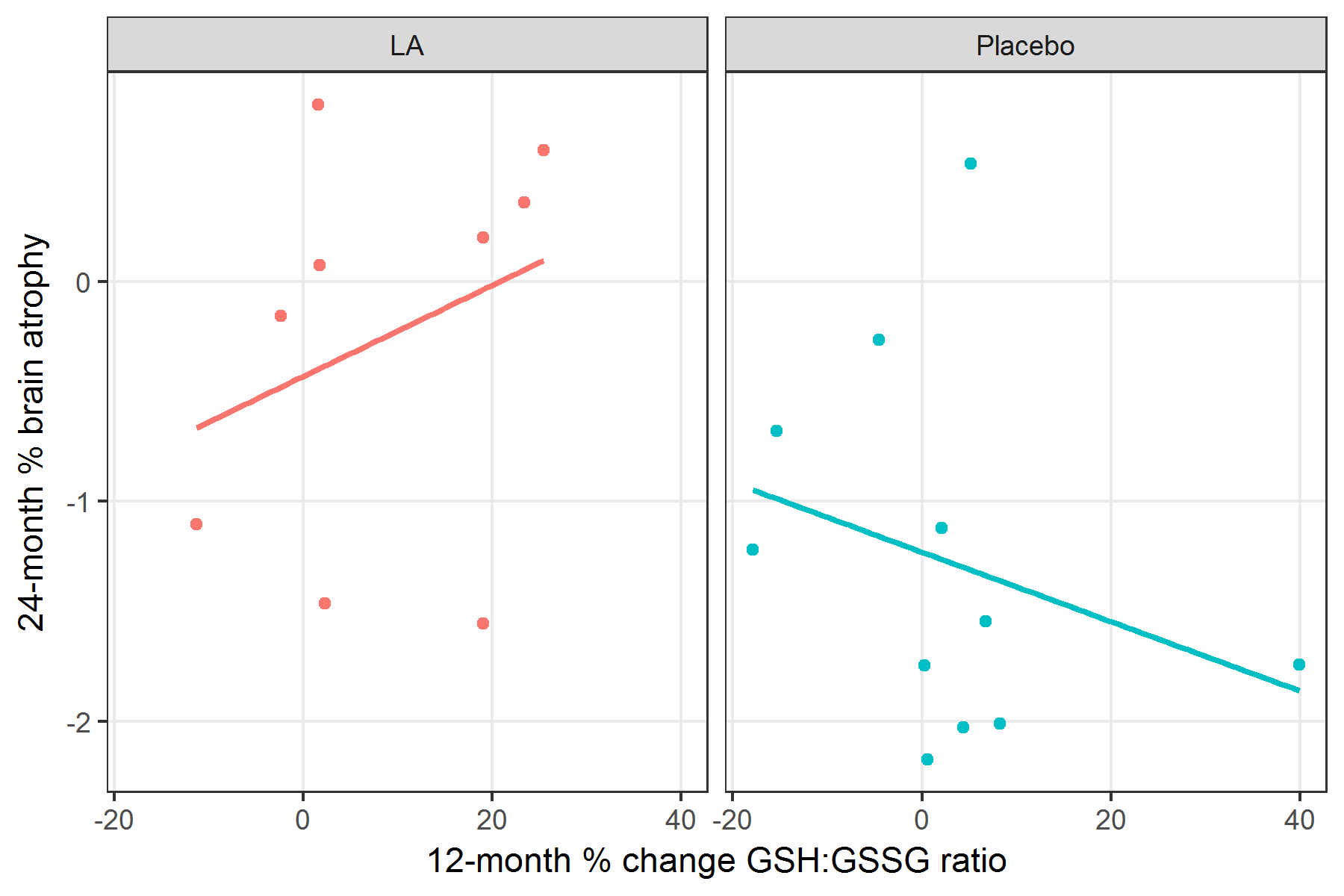
The model for Aim 2 will be a linear regression model.

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   
## -1.28391 -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   
## pctChangeConcRatio -0.001996 0.014784 -0.135 0.8942   
## studyArmPlacebo -1.035179 0.410478 -2.522 0.0219 \*  
## ---  
## 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 ~ pctChangeConcRatio + studyArm + pctChangeConcRatio:studyArm,   
## data = df2)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -1.5167 -0.5468 0.1300 0.3598 1.8464   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -0.43090 0.35922 -1.200 0.2478   
## pctChangeConcRatio 0.02071 0.02372 0.873 0.3957   
## studyArmPlacebo -0.79917 0.44921 -1.779 0.0942 .  
## pctChangeConcRatio:studyArmPlacebo -0.03649 0.03008 -1.213 0.2427   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.8791 on 16 degrees of freedom  
## Multiple R-squared: 0.3387, Adjusted R-squared: 0.2147   
## F-statistic: 2.731 on 3 and 16 DF, p-value: 0.07818

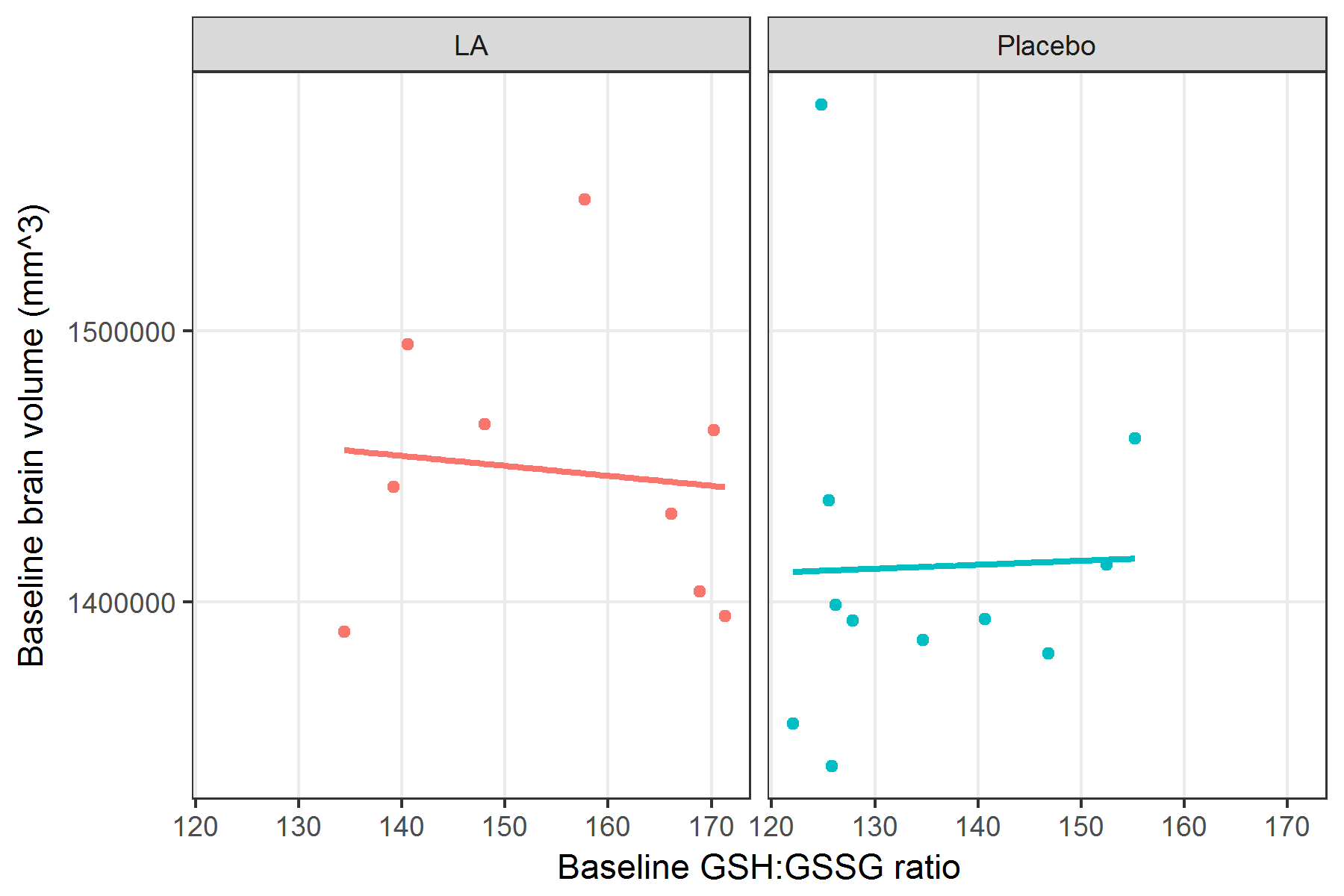
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | p.value |
| (Intercept) | -0.43 | 0.36 | -1.20 | 0.2478 |
| pctChangeConcRatio | 0.02 | 0.02 | 0.87 | 0.3957 |
| studyArmPlacebo | -0.80 | 0.45 | -1.78 | 0.0942 |
| pctChangeConcRatio:studyArmPlacebo | -0.04 | 0.03 | -1.21 | 0.2427 |

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 -0.002 (p-value = 0.894)
  + Partial correlation coefficient = -0.0327
  + GSH:GSSG concentration change explains 0.107% 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   
## -74629 -33266 -14741 18701 168892   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) 1469063 169843 8.650 1.24e-07 \*\*\*  
## concRatioM0 -134 1086 -0.123 0.903   
## studyArmPlacebo -38074 35487 -1.073 0.298   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 61610 on 17 degrees of freedom  
## Multiple R-squared: 0.08816, Adjusted R-squared: -0.01911   
## F-statistic: 0.8218 on 2 and 17 DF, p-value: 0.4564

### 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 -134 (p-value = 0.903)
  + Partial correlation coefficient = -0.0299
  + Baseline GSH:GSSG concentration ratio explains 0.0894% of the variation in brain atrophy after adjusting for study arm