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

Benjamin Chan (chanb@ohsu.edu)

2018-03-14

# Import raw data

Joined data set. Include only the

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

## 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 ...

## 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

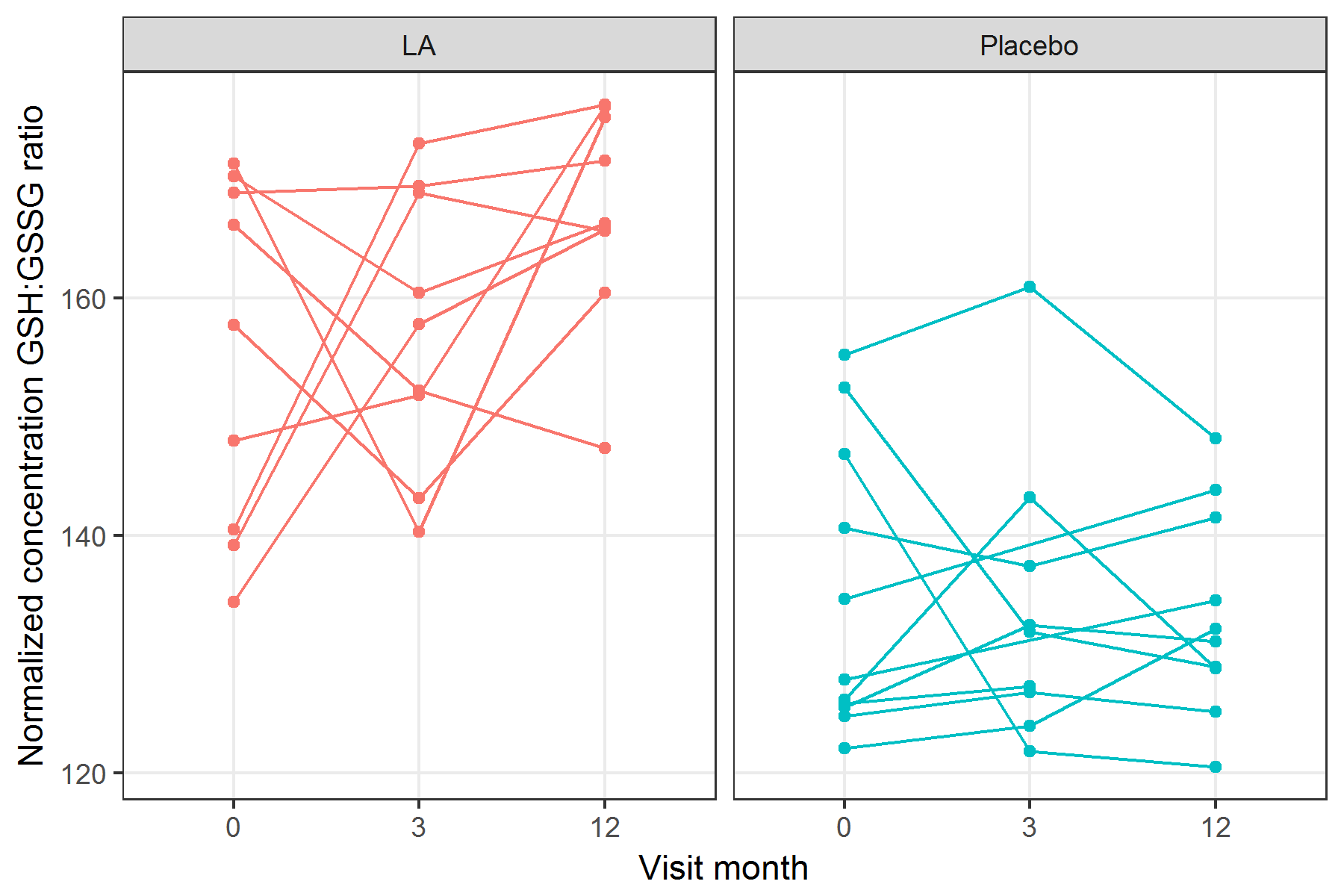
Sample 44 = 143 M0  
Sample 45 = 143 M3  
Sample 46 = 143 M12

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 |

# Aim 1

## Normalized GSH:GSSG concentration ratio



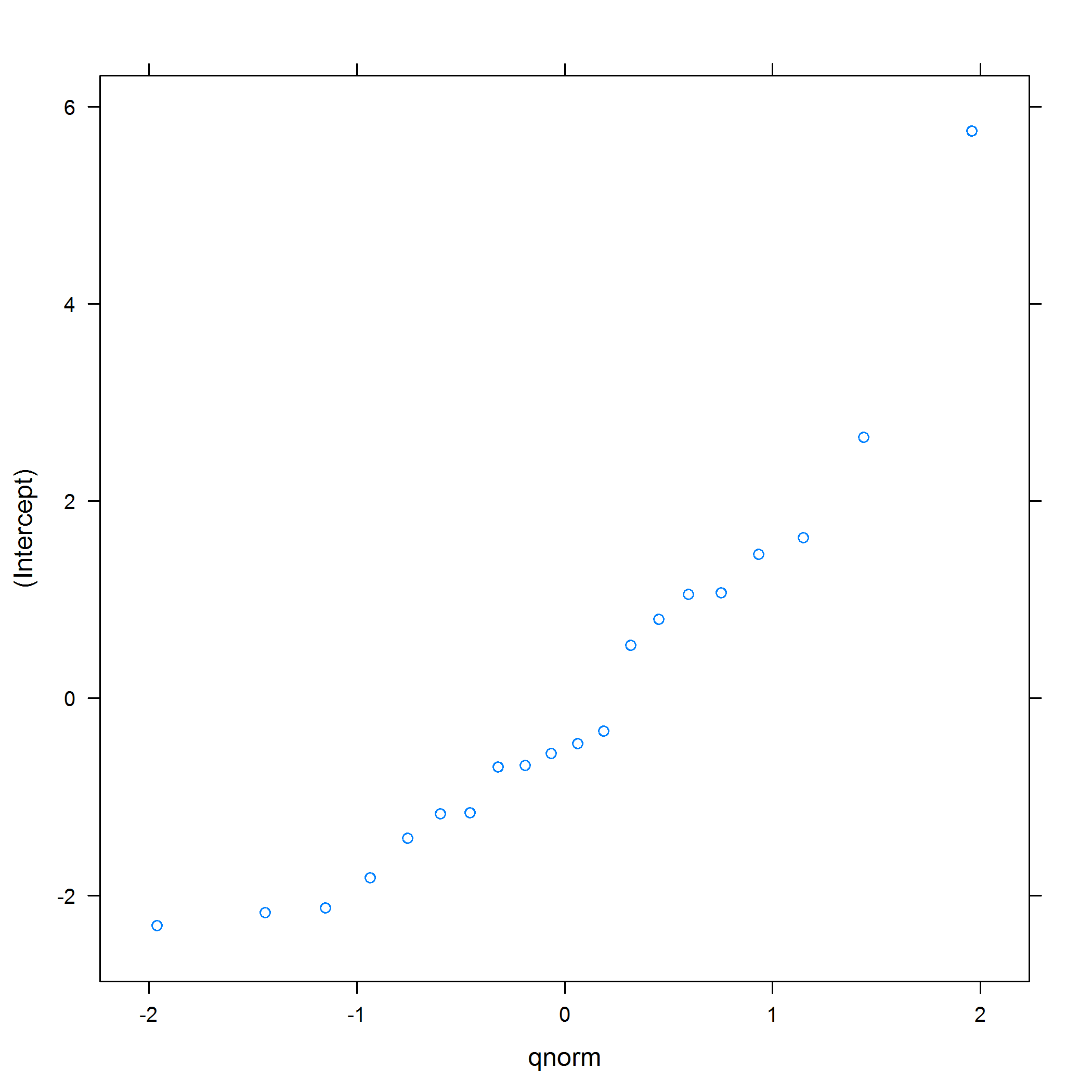
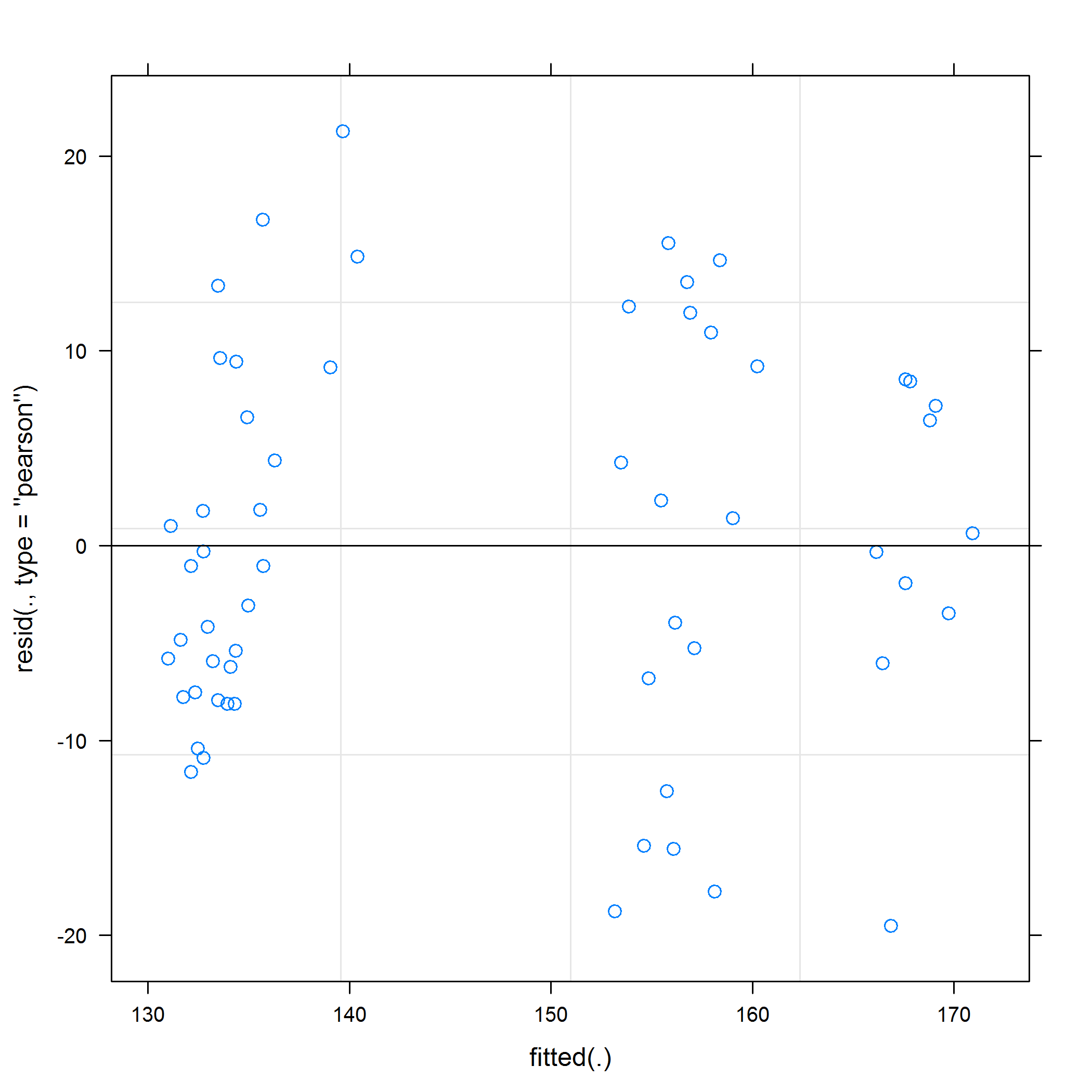
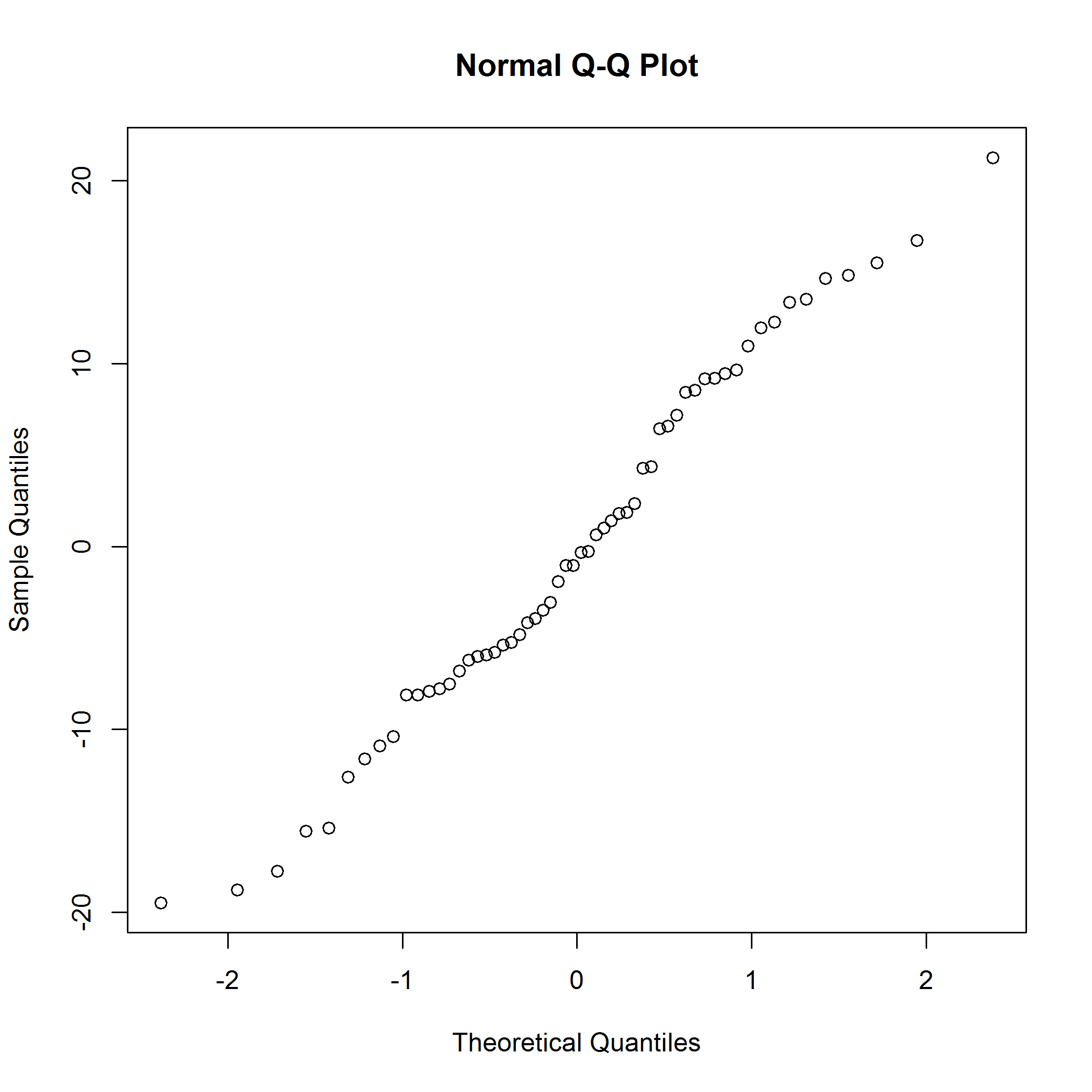
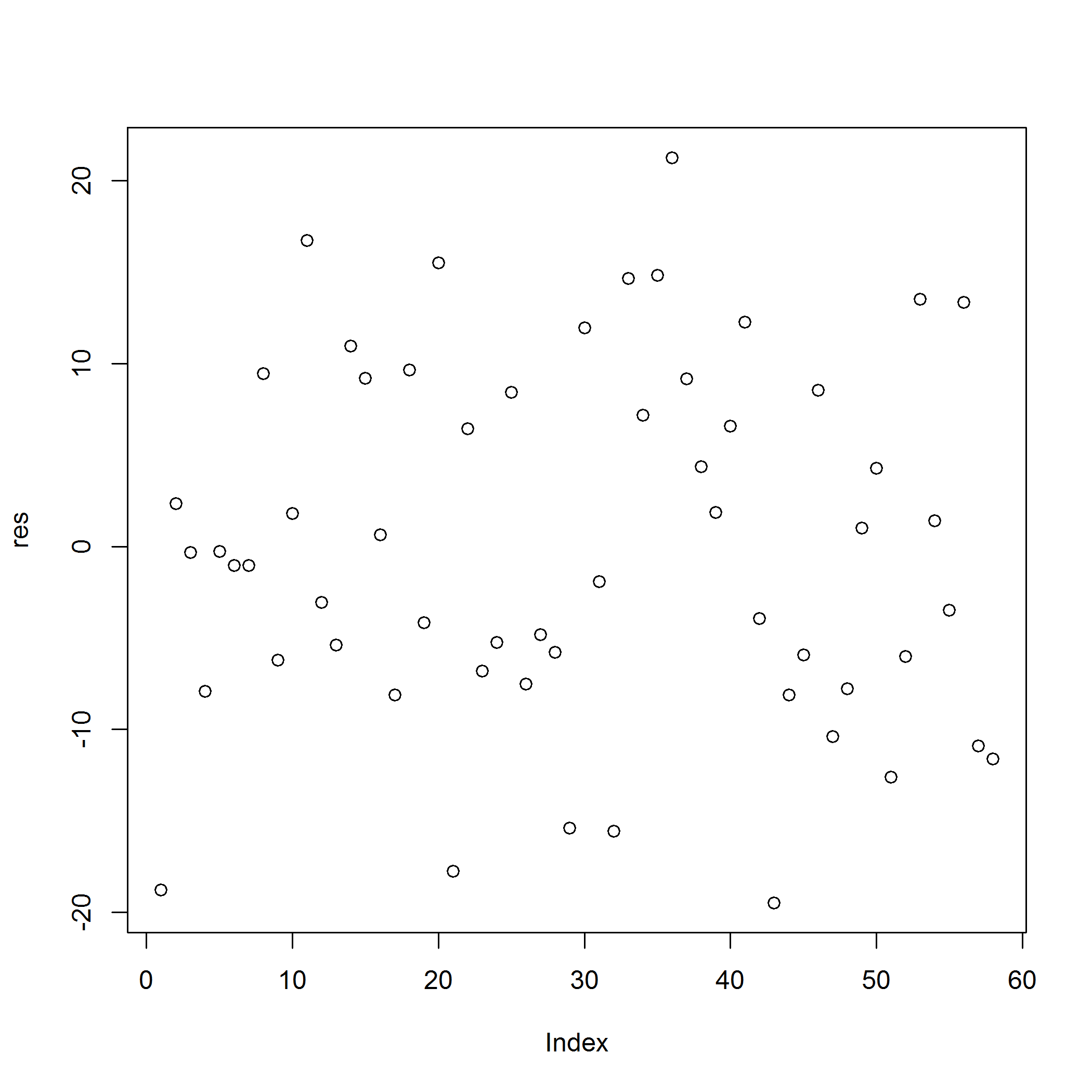
figures/lineplotNormalizedConcRatio.png

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | group |
| (Intercept) | 155.30 | 3.84 | 40.45 | fixed |
| studyArmPlacebo | -20.65 | 5.17 | -3.99 | fixed |
| visitMonth3 | 2.29 | 5.12 | 0.45 | fixed |
| visitMonth12 | 12.99 | 5.00 | 2.60 | fixed |
| studyArmPlacebo:visitMonth3 | -3.01 | 7.09 | -0.42 | fixed |
| studyArmPlacebo:visitMonth12 | -14.34 | 6.91 | -2.07 | fixed |
| sd\_(Intercept).patientID | 3.87 | NA | NA | patientID |
| sd\_Observation.Residual | 10.86 | NA | NA | Residual |

## Linear mixed model fit by REML ['lmerMod']  
## Formula: normalizedConcRatio ~ studyArm \* visitMonth + (1 | patientID)  
## Data: df  
##   
## REML criterion at convergence: 414.8  
##   
## Scaled residuals:   
## Min 1Q Median 3Q Max   
## -1.79662 -0.61361 -0.06285 0.78467 1.95927   
##   
## Random effects:  
## Groups Name Variance Std.Dev.  
## patientID (Intercept) 14.97 3.869   
## Residual 117.86 10.856   
## Number of obs: 58, groups: patientID, 20  
##   
## Fixed effects:  
## Estimate Std. Error t value  
## (Intercept) 155.296 3.839 40.45  
## studyArmPlacebo -20.646 5.174 -3.99  
## visitMonth3 2.285 5.118 0.45  
## visitMonth12 12.988 5.001 2.60  
## studyArmPlacebo:visitMonth3 -3.010 7.088 -0.42  
## studyArmPlacebo:visitMonth12 -14.341 6.914 -2.07  
##   
## Correlation of Fixed Effects:  
## (Intr) stdyAP vstMn3 vstM12 sAP:M3  
## stdyArmPlcb -0.741   
## visitMonth3 -0.666 0.495   
## visitMnth12 -0.690 0.517 0.512   
## stdyArmP:M3 0.481 -0.649 -0.722 -0.369   
## stdyArP:M12 0.499 -0.673 -0.370 -0.726 0.485

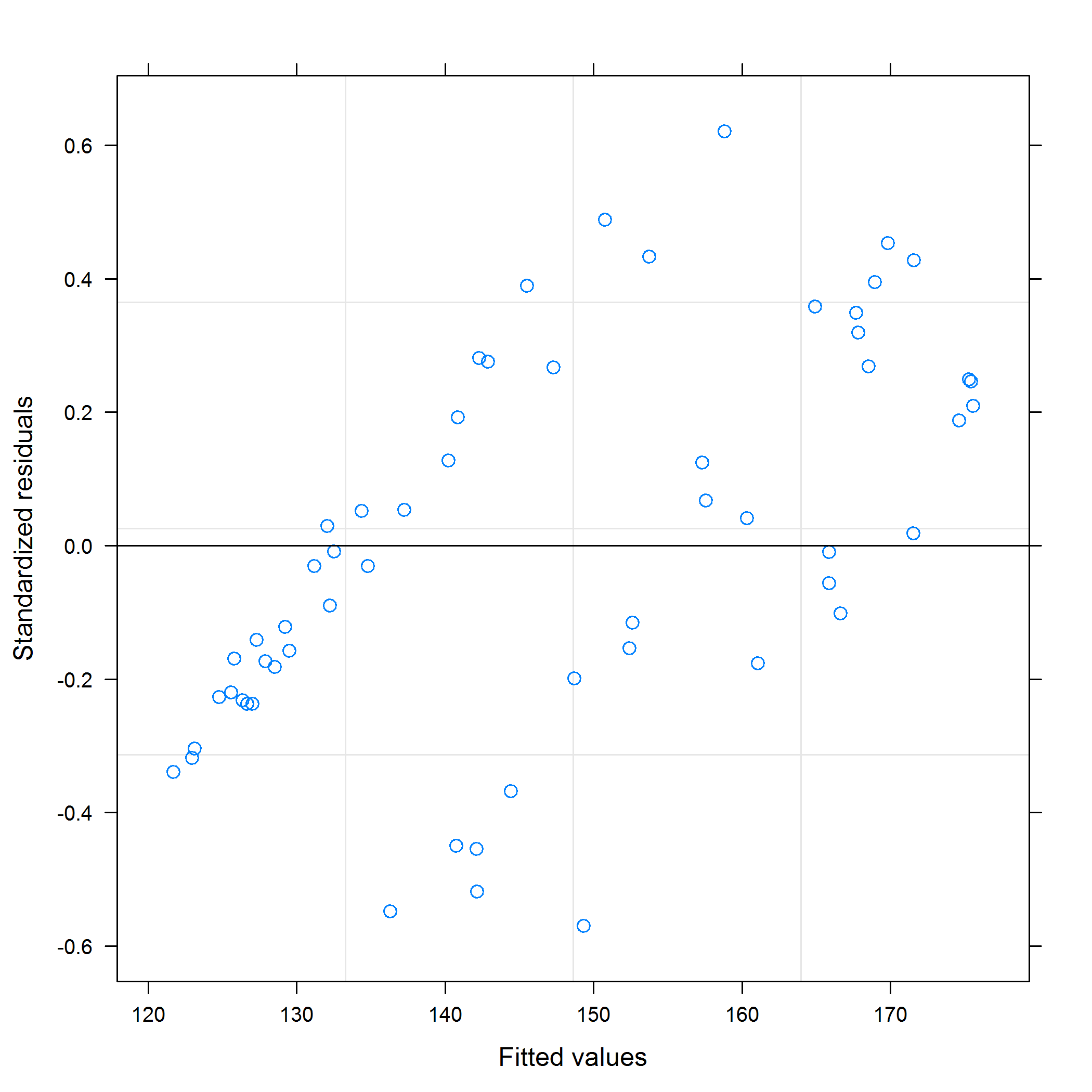
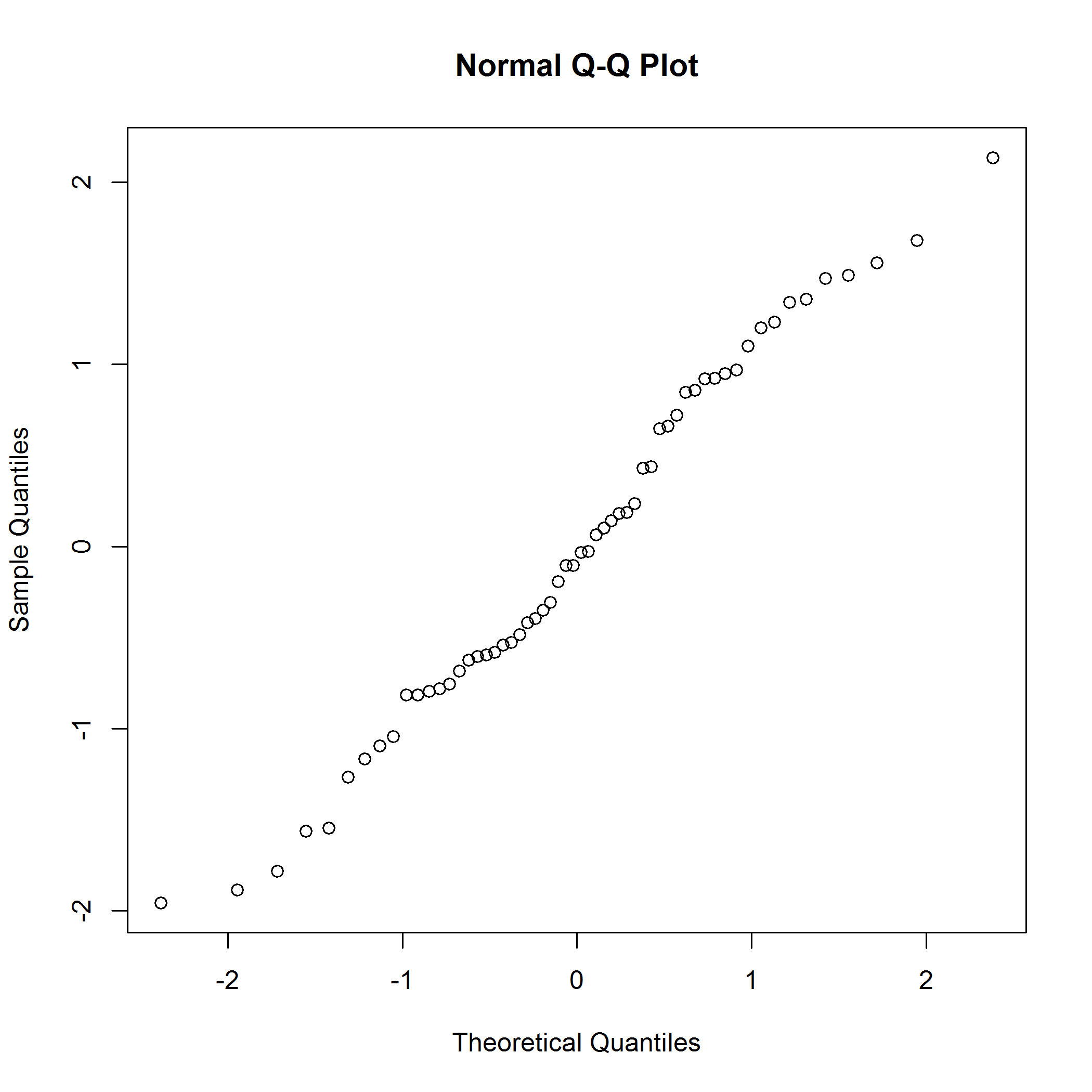
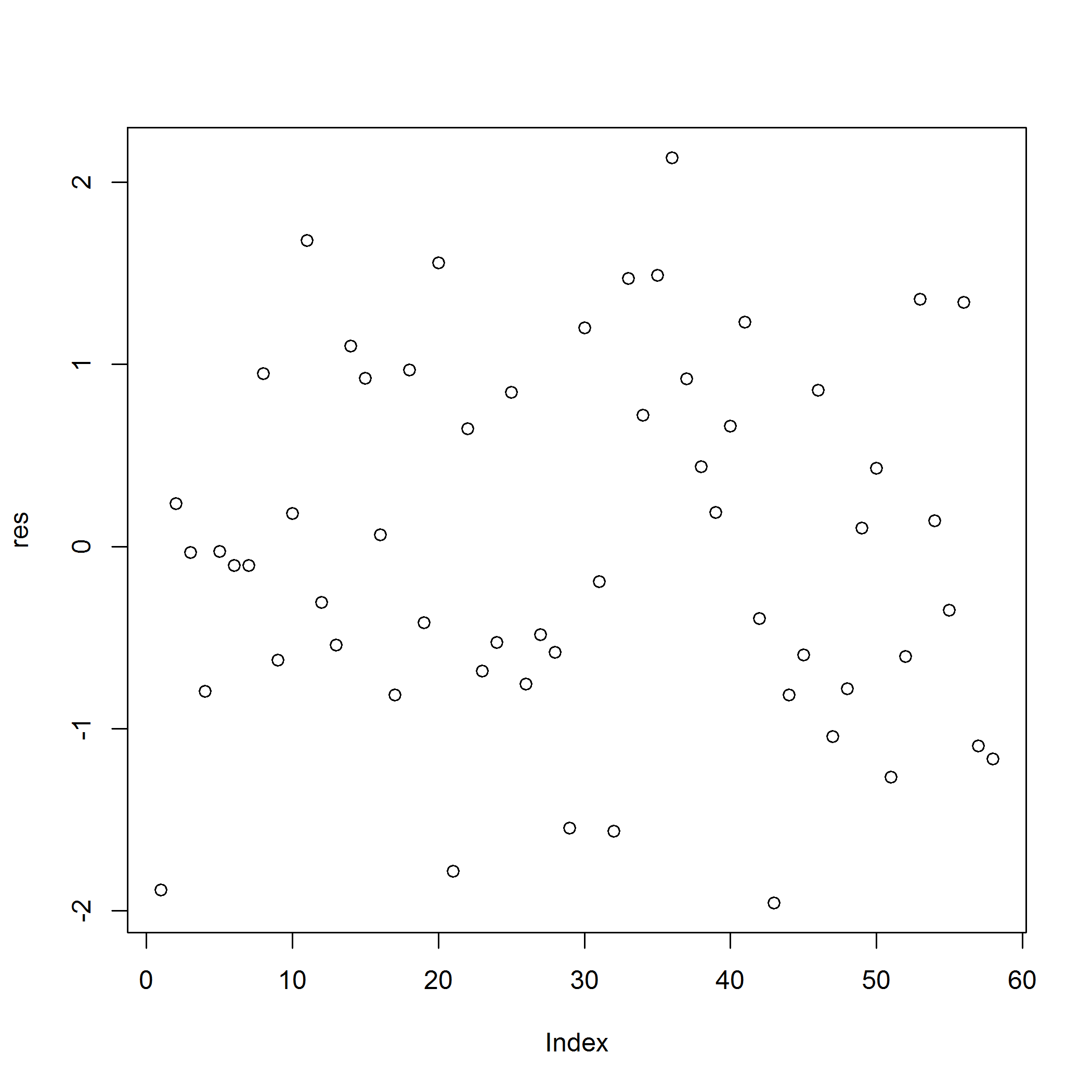
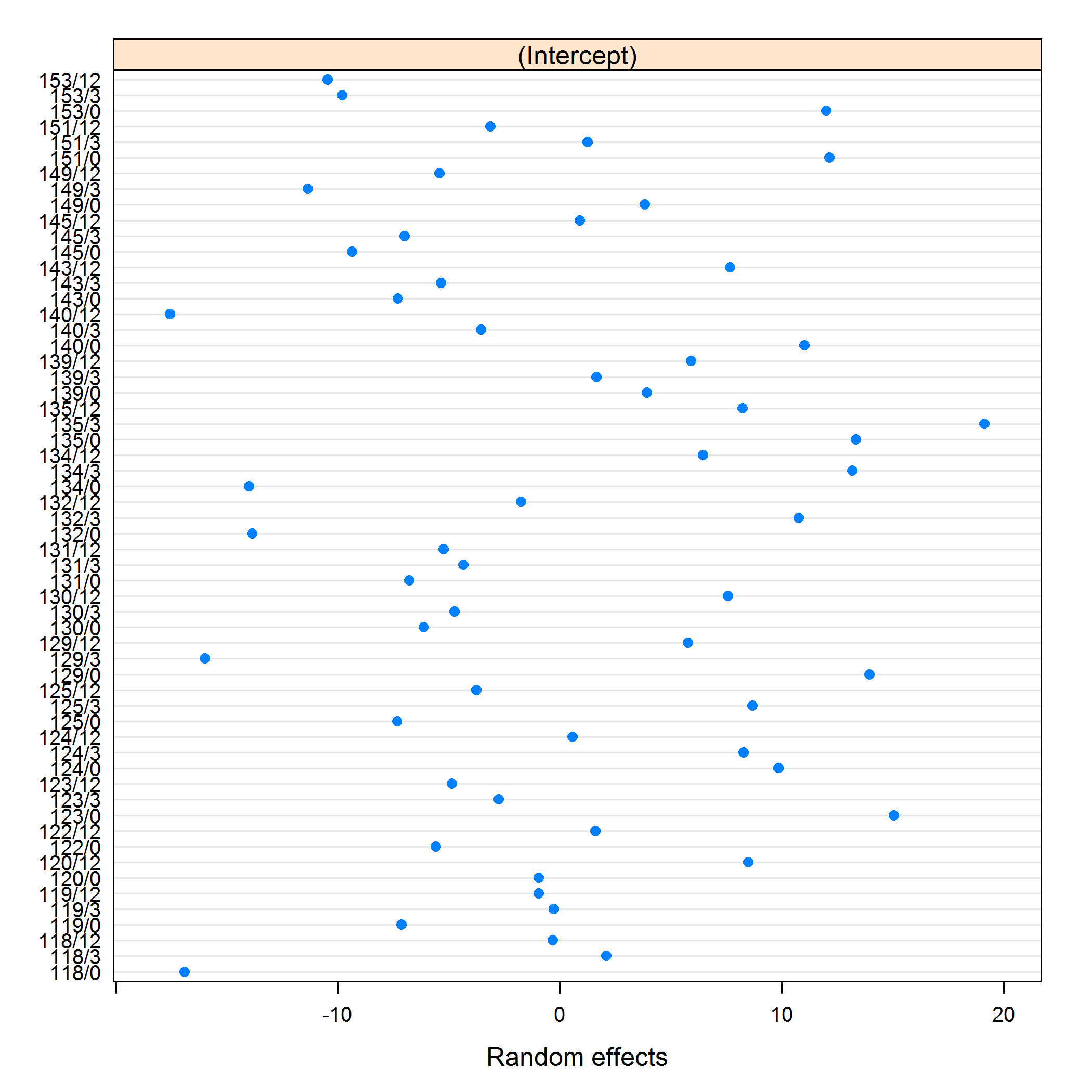
## $patientID

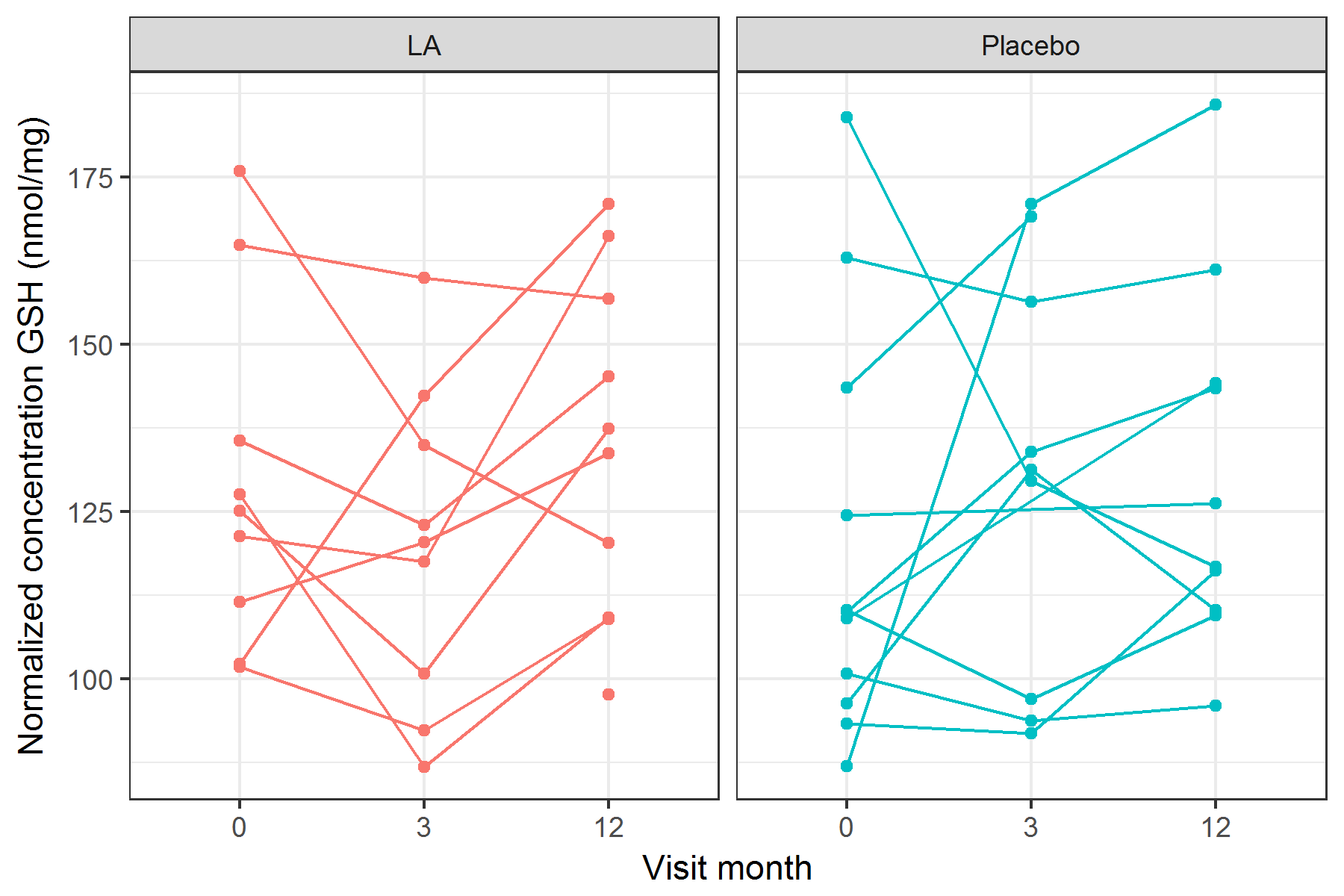
Mixed effects model using the **nlme** package.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | p.value |
| (Intercept) | 155.30 | 3.84 | 40.45 | 0.0000 |
| studyArmPlacebo | -20.65 | 5.17 | -3.99 | 0.0003 |
| visitMonth3 | 2.29 | 5.12 | 0.45 | 0.6581 |
| visitMonth12 | 12.99 | 5.00 | 2.60 | 0.0139 |
| studyArmPlacebo:visitMonth3 | -3.01 | 7.09 | -0.42 | 0.6739 |
| studyArmPlacebo:visitMonth12 | -14.34 | 6.91 | -2.07 | 0.0459 |

## Linear mixed-effects model fit by REML  
## Data: df   
## AIC BIC logLik  
## 434.8007 454.3131 -207.4003  
##   
## Random effects:  
## Formula: ~1 | patientID  
## (Intercept)  
## StdDev: 3.869342  
##   
## Formula: ~1 | visitMonth %in% patientID  
## (Intercept) Residual  
## StdDev: 10.29691 3.439829  
##   
## Correlation Structure: AR(1)  
## Formula: ~1 | patientID/visitMonth   
## Parameter estimate(s):  
## Phi   
## 0   
## Fixed effects: normalizedConcRatio ~ studyArm \* visitMonth   
## Value Std.Error DF t-value p-value  
## (Intercept) 155.29638 3.839251 33 40.44966 0.0000  
## studyArmPlacebo -20.64606 5.174099 33 -3.99027 0.0003  
## visitMonth3 2.28511 5.117698 33 0.44651 0.6581  
## visitMonth12 12.98775 5.000699 33 2.59719 0.0139  
## studyArmPlacebo:visitMonth3 -3.00959 7.088158 33 -0.42459 0.6739  
## studyArmPlacebo:visitMonth12 -14.34077 6.914091 33 -2.07414 0.0459  
## Correlation:   
## (Intr) stdyAP vstMn3 vstM12 sAP:M3  
## studyArmPlacebo -0.741   
## visitMonth3 -0.666 0.495   
## visitMonth12 -0.690 0.517 0.512   
## studyArmPlacebo:visitMonth3 0.481 -0.649 -0.722 -0.369   
## studyArmPlacebo:visitMonth12 0.499 -0.673 -0.370 -0.726 0.485  
##   
## Standardized Within-Group Residuals:  
## Min Q1 Med Q3 Max   
## -0.56926116 -0.19442183 -0.01991562 0.24862441 0.62079739   
##   
## Number of Observations: 58  
## Number of Groups:   
## patientID visitMonth %in% patientID   
## 20 58



## Normalized concentration GSH



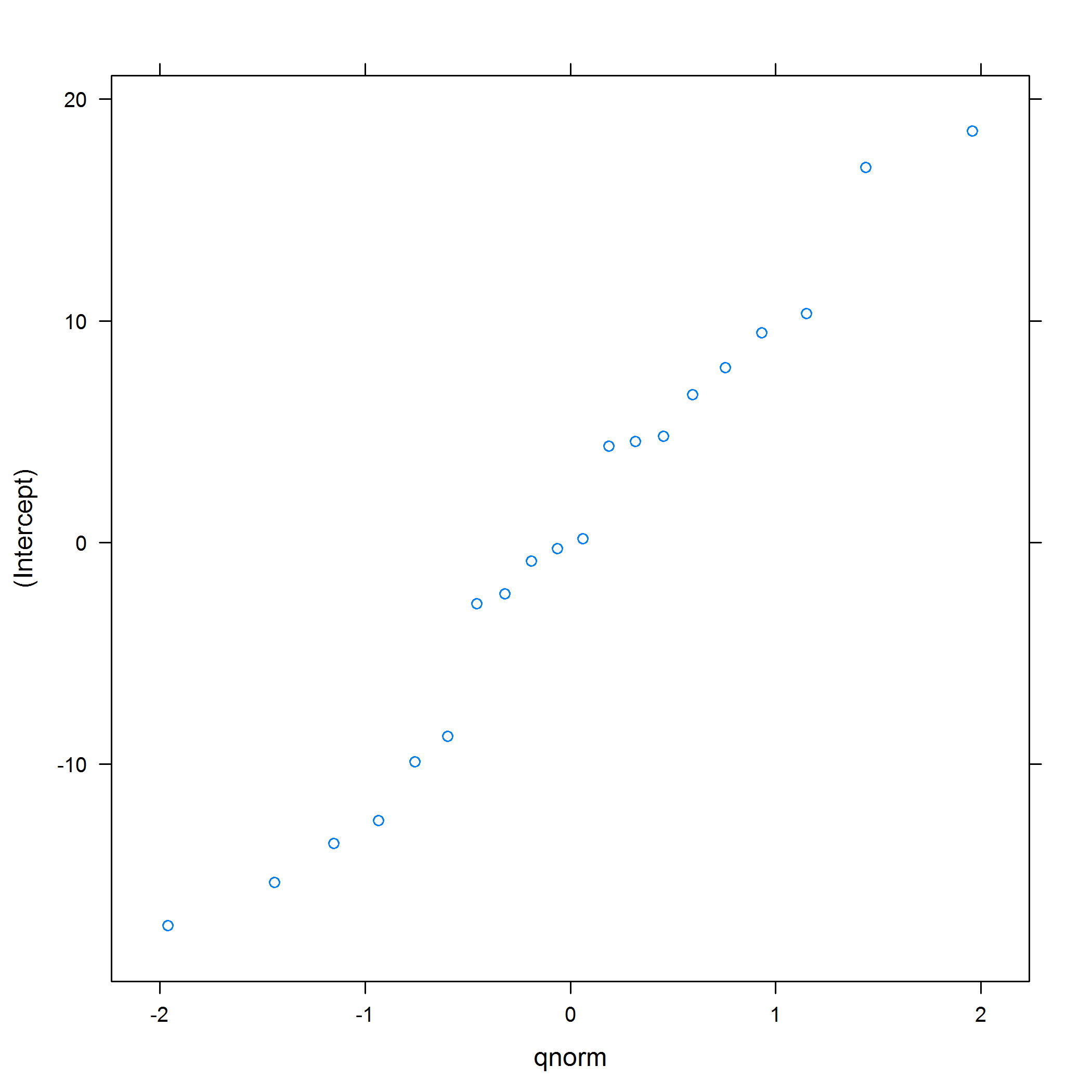
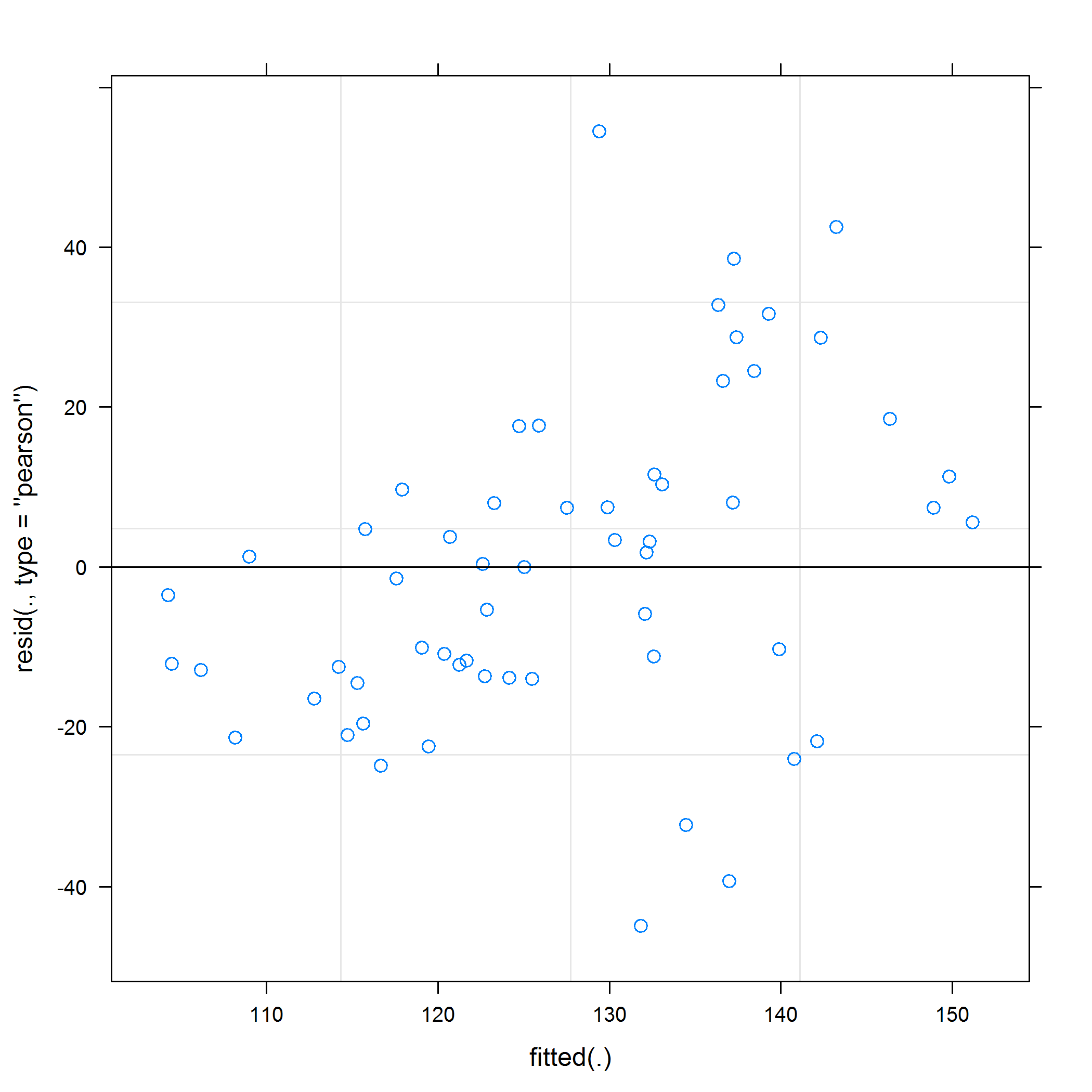
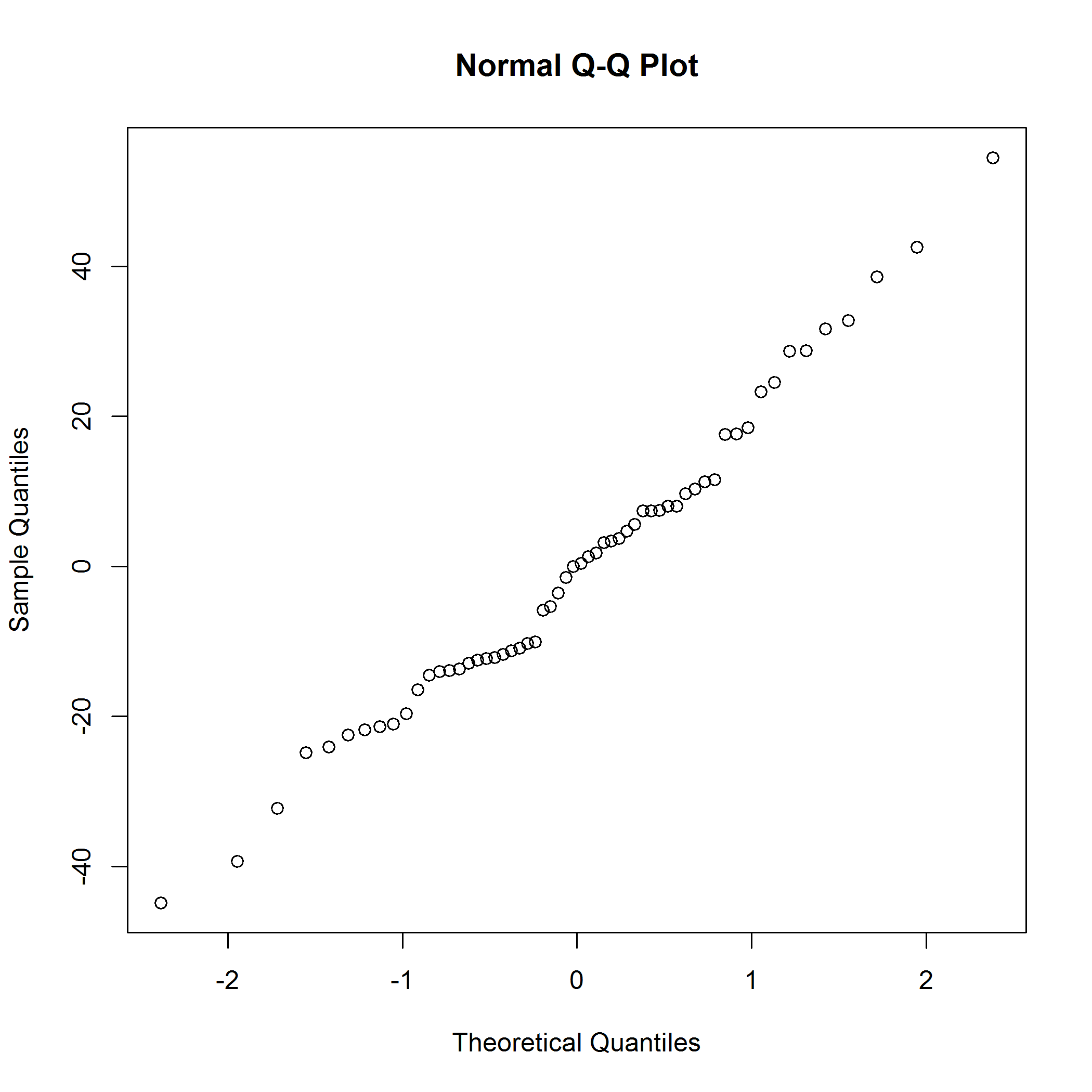
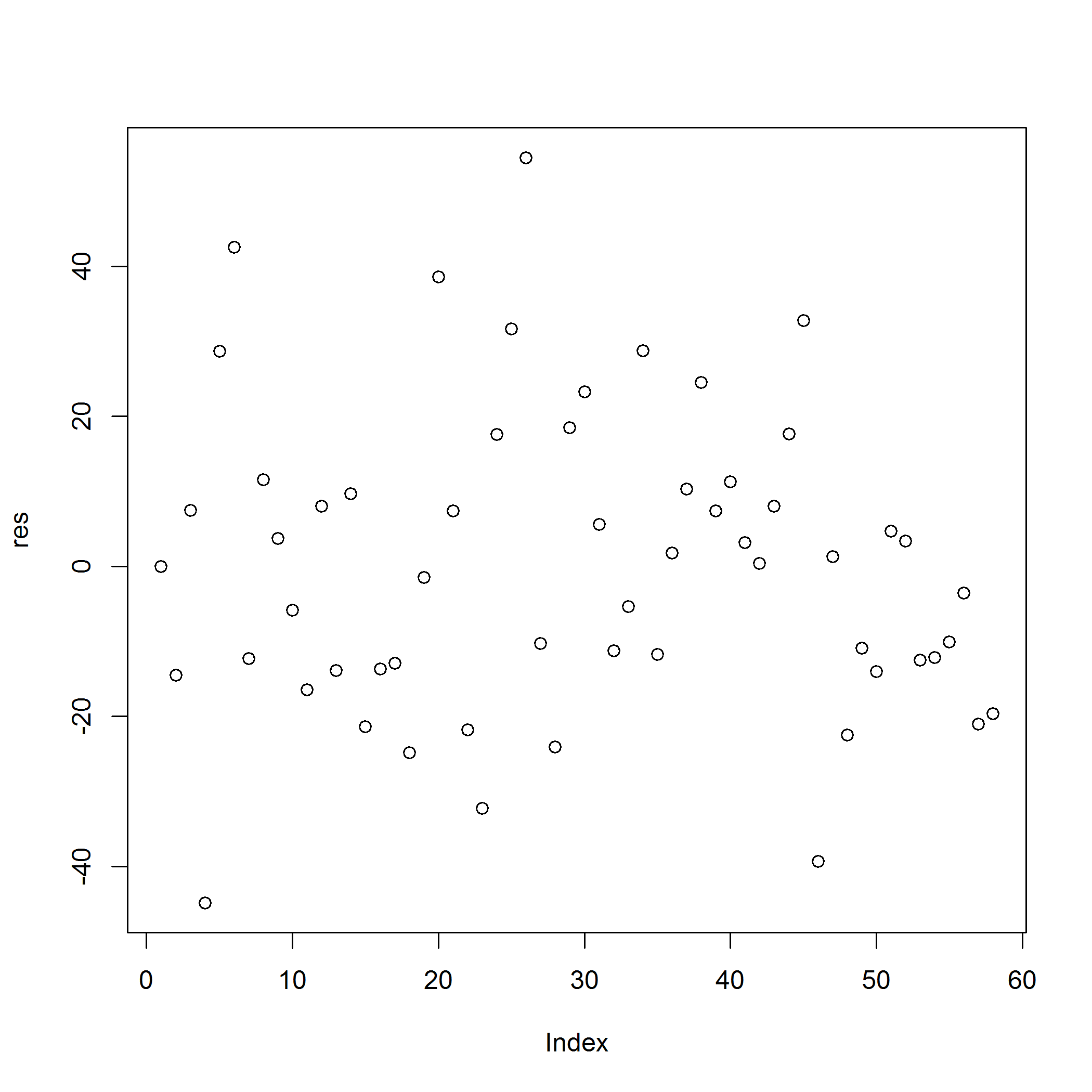
figures/lineplotNormalizedConcGSH.png

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | group |
| (Intercept) | 127.81 | 9.18 | 13.92 | fixed |
| studyArmPlacebo | -6.28 | 12.34 | -0.51 | fixed |
| visitMonth3 | -9.74 | 11.06 | -0.88 | fixed |
| visitMonth12 | 4.84 | 10.84 | 0.45 | fixed |
| studyArmPlacebo:visitMonth3 | 20.22 | 15.36 | 1.32 | fixed |
| studyArmPlacebo:visitMonth12 | 6.54 | 15.01 | 0.44 | fixed |
| sd\_(Intercept).patientID | 14.68 | NA | NA | patientID |
| sd\_Observation.Residual | 23.46 | NA | NA | Residual |

## Linear mixed model fit by REML ['lmerMod']  
## Formula: normalizedConcGSH ~ studyArm \* visitMonth + (1 | patientID)  
## Data: df  
##   
## REML criterion at convergence: 502.9  
##   
## Scaled residuals:   
## Min 1Q Median 3Q Max   
## -1.91355 -0.57401 0.00879 0.43321 2.32305   
##   
## Random effects:  
## Groups Name Variance Std.Dev.  
## patientID (Intercept) 215.5 14.68   
## Residual 550.4 23.46   
## Number of obs: 58, groups: patientID, 20  
##   
## Fixed effects:  
## Estimate Std. Error t value  
## (Intercept) 127.808 9.184 13.916  
## studyArmPlacebo -6.278 12.339 -0.509  
## visitMonth3 -9.741 11.059 -0.881  
## visitMonth12 4.842 10.836 0.447  
## studyArmPlacebo:visitMonth3 20.222 15.360 1.316  
## studyArmPlacebo:visitMonth12 6.538 15.007 0.436  
##   
## Correlation of Fixed Effects:  
## (Intr) stdyAP vstMn3 vstM12 sAP:M3  
## stdyArmPlcb -0.739   
## visitMonth3 -0.602 0.448   
## visitMnth12 -0.634 0.483 0.510   
## stdyArmP:M3 0.434 -0.588 -0.720 -0.366   
## stdyArP:M12 0.455 -0.616 -0.368 -0.728 0.480

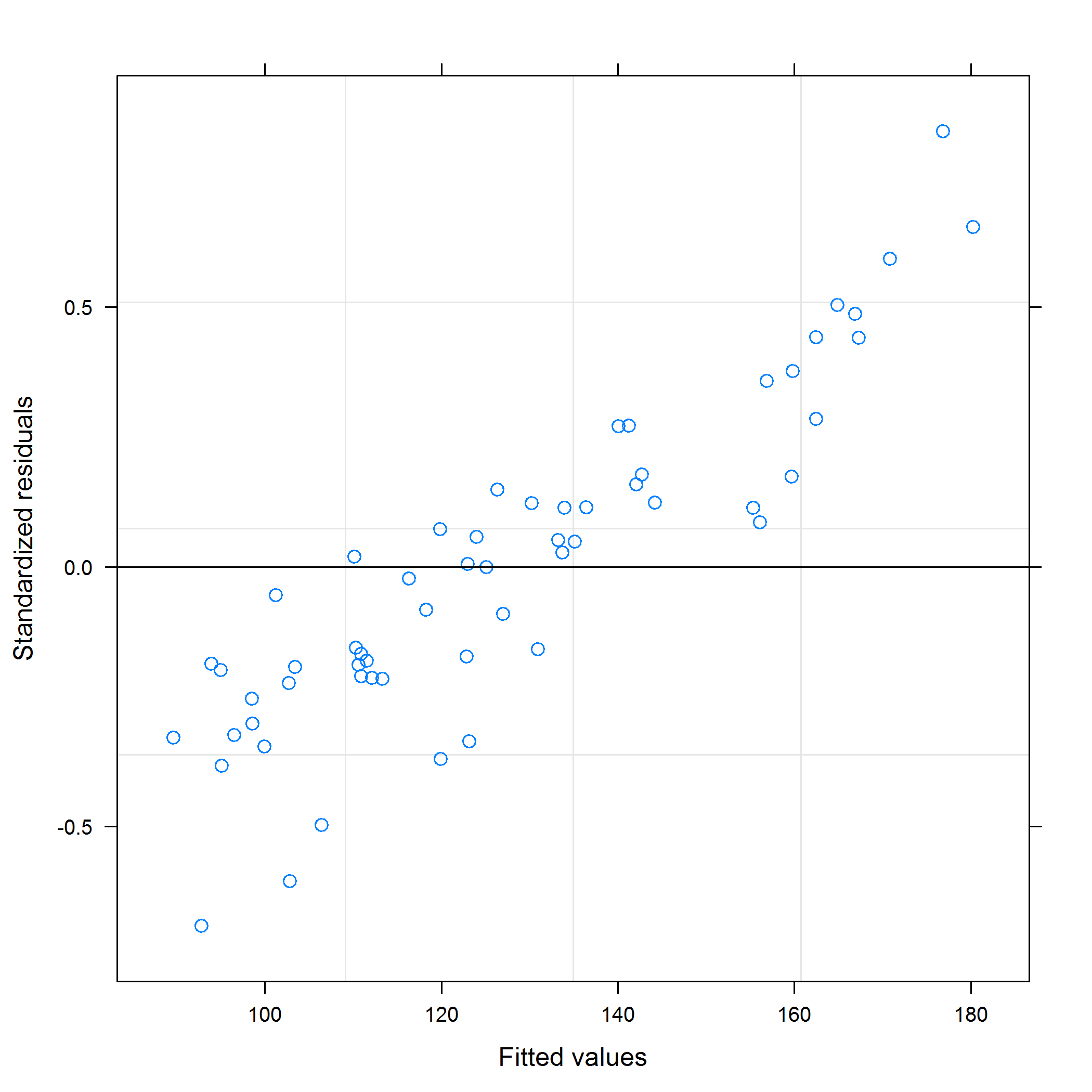
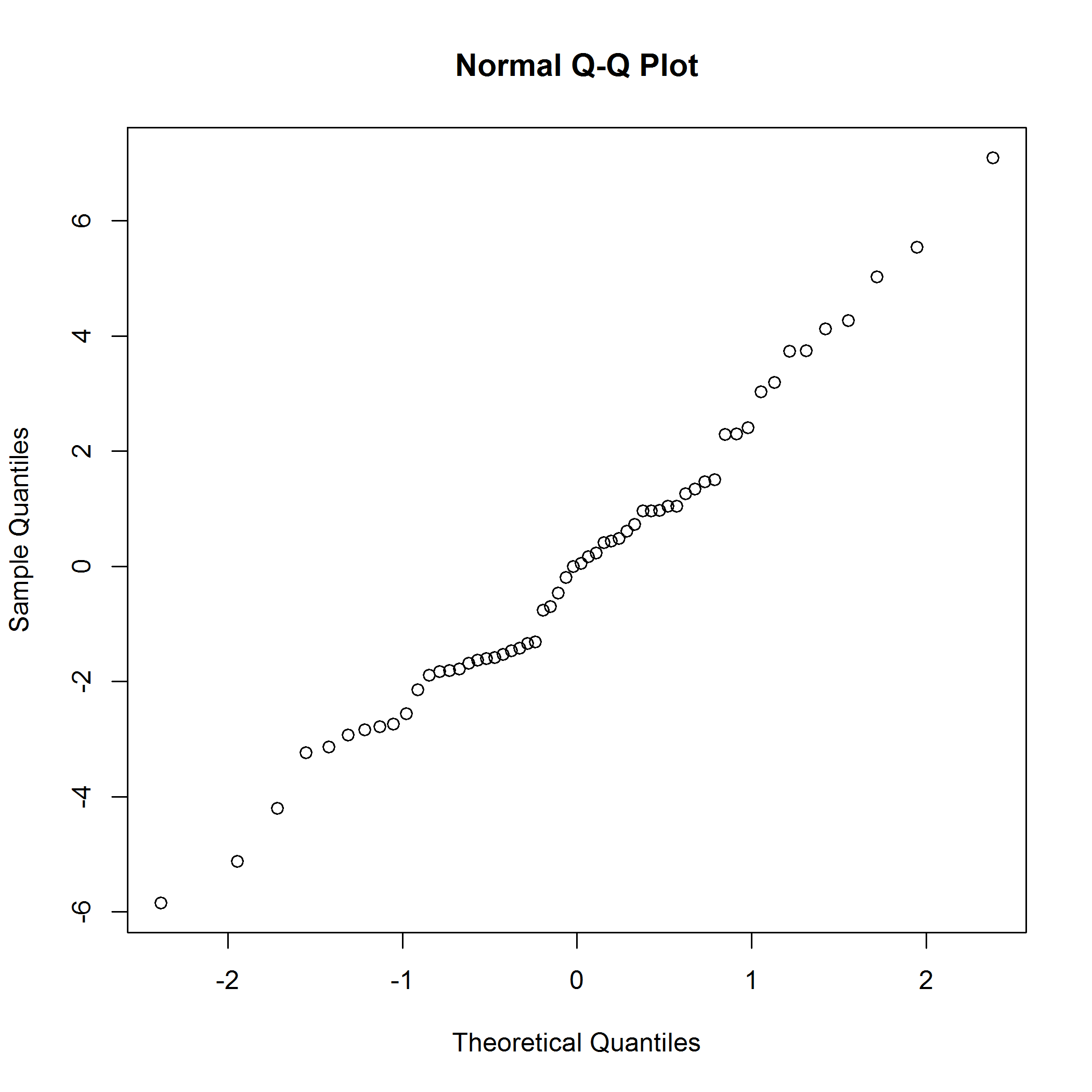
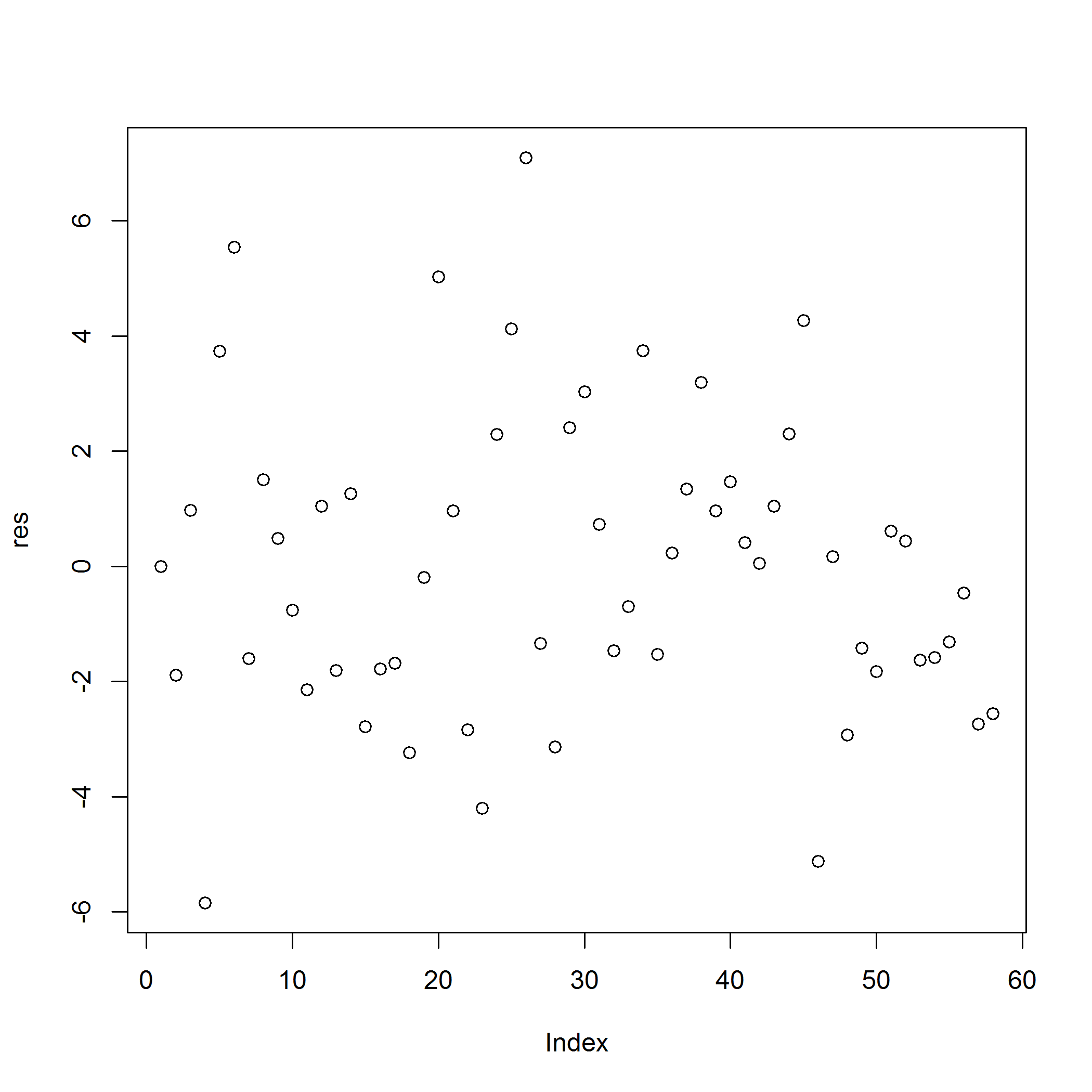
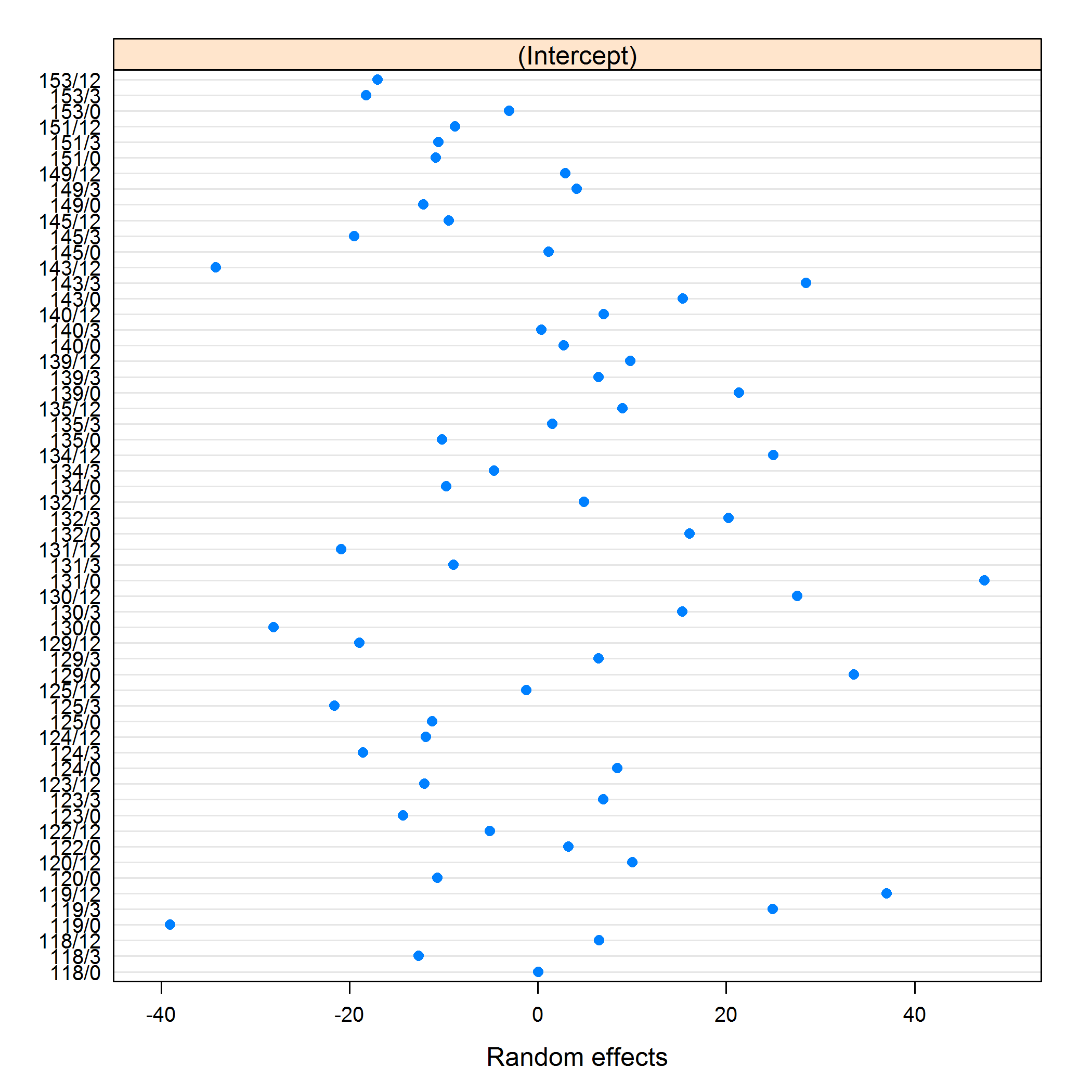
## $patientID

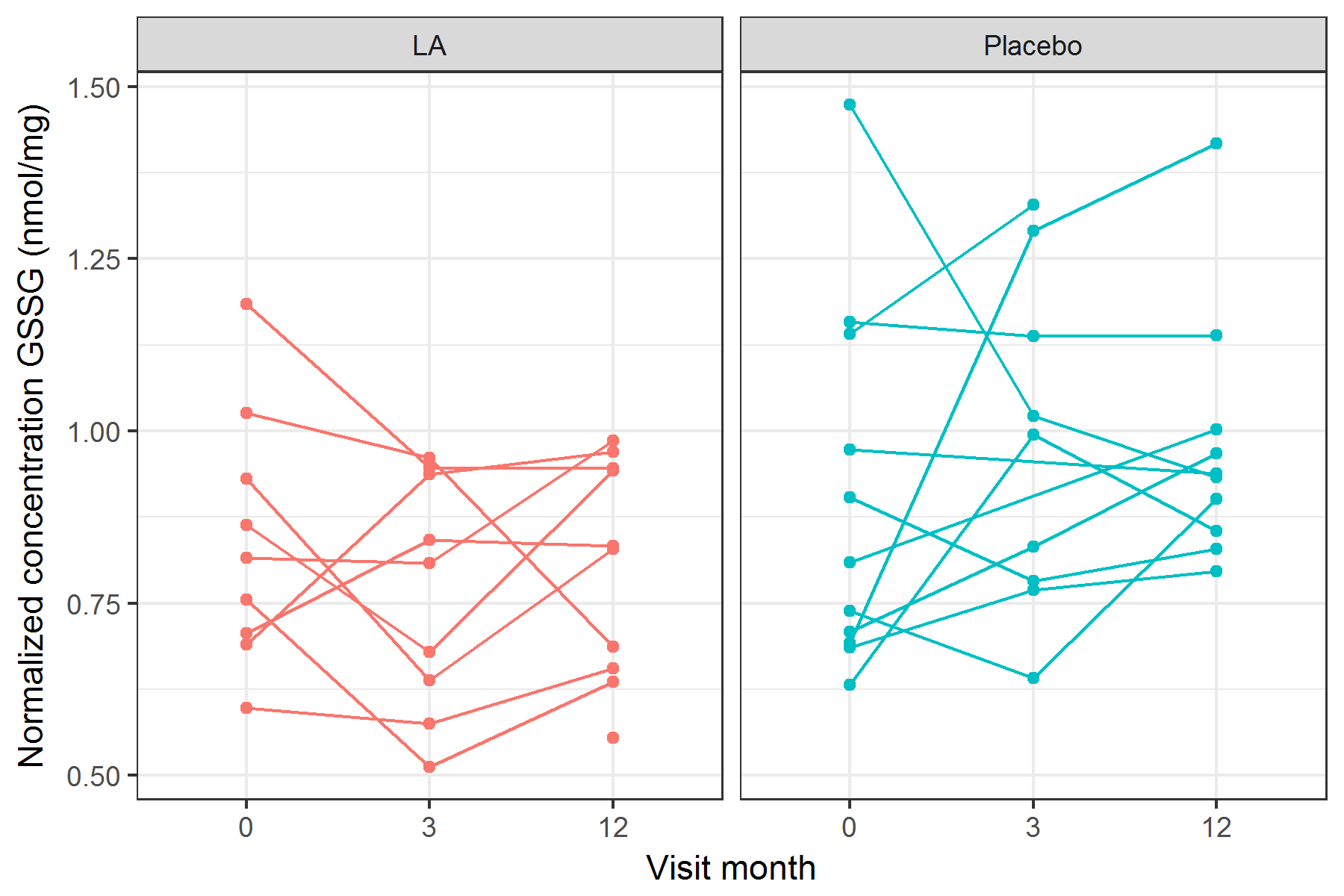
Mixed effects model using the **nlme** package.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | p.value |
| (Intercept) | 127.81 | 9.18 | 13.92 | 0.0000 |
| studyArmPlacebo | -6.28 | 12.34 | -0.51 | 0.6143 |
| visitMonth3 | -9.74 | 11.06 | -0.88 | 0.3848 |
| visitMonth12 | 4.84 | 10.84 | 0.45 | 0.6579 |
| studyArmPlacebo:visitMonth3 | 20.22 | 15.36 | 1.32 | 0.1971 |
| studyArmPlacebo:visitMonth12 | 6.54 | 15.01 | 0.44 | 0.6659 |

## Linear mixed-effects model fit by REML  
## Data: df   
## AIC BIC logLik  
## 522.9283 542.4407 -251.4641  
##   
## Random effects:  
## Formula: ~1 | patientID  
## (Intercept)  
## StdDev: 14.67971  
##   
## Formula: ~1 | visitMonth %in% patientID  
## (Intercept) Residual  
## StdDev: 21.87972 8.465757  
##   
## Correlation Structure: AR(1)  
## Formula: ~1 | patientID/visitMonth   
## Parameter estimate(s):  
## Phi   
## 0   
## Fixed effects: normalizedConcGSH ~ studyArm \* visitMonth   
## Value Std.Error DF t-value p-value  
## (Intercept) 127.80760 9.184201 33 13.916029 0.0000  
## studyArmPlacebo -6.27833 12.338916 33 -0.508823 0.6143  
## visitMonth3 -9.74082 11.059349 33 -0.880777 0.3848  
## visitMonth12 4.84189 10.836250 33 0.446823 0.6579  
## studyArmPlacebo:visitMonth3 20.22194 15.360464 33 1.316493 0.1971  
## studyArmPlacebo:visitMonth12 6.53845 15.007012 33 0.435693 0.6659  
## Correlation:   
## (Intr) stdyAP vstMn3 vstM12 sAP:M3  
## studyArmPlacebo -0.739   
## visitMonth3 -0.602 0.448   
## visitMonth12 -0.634 0.483 0.510   
## studyArmPlacebo:visitMonth3 0.434 -0.588 -0.720 -0.366   
## studyArmPlacebo:visitMonth12 0.455 -0.616 -0.368 -0.728 0.480  
##   
## Standardized Within-Group Residuals:  
## Min Q1 Med Q3 Max   
## -0.690509071 -0.207131458 0.003173197 0.156324523 0.838280113   
##   
## Number of Observations: 58  
## Number of Groups:   
## patientID visitMonth %in% patientID   
## 20 58



## Normalized concentration GSSG



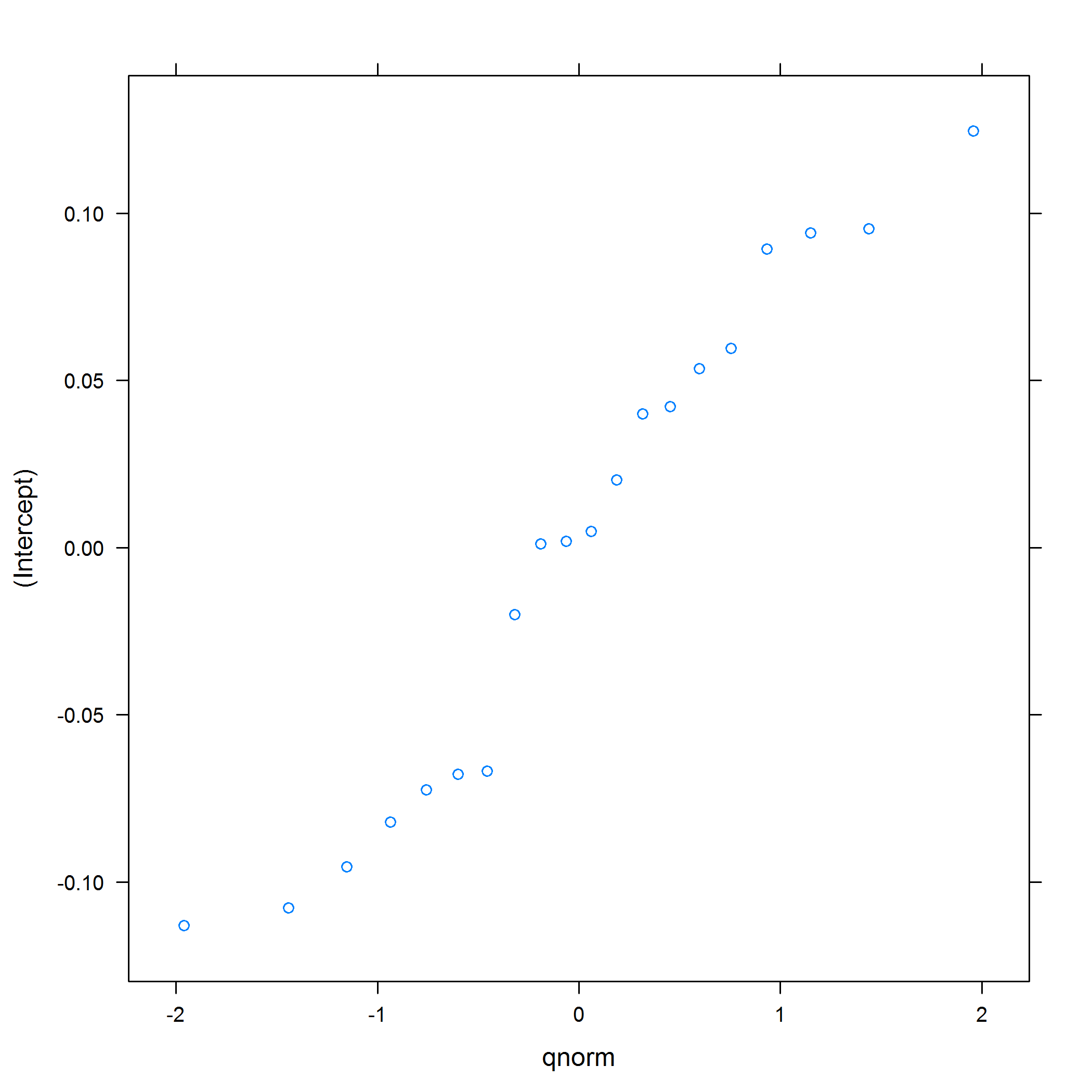
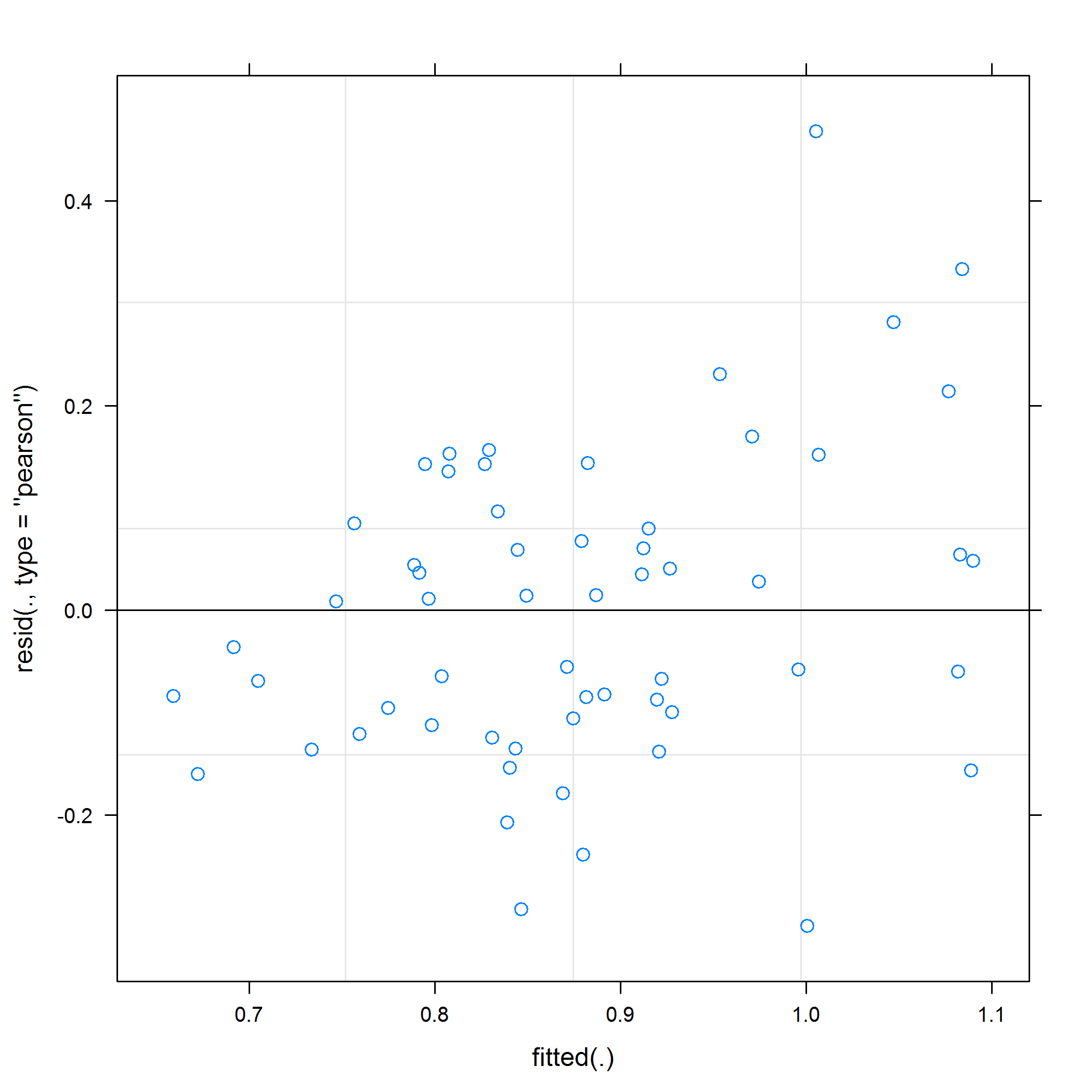
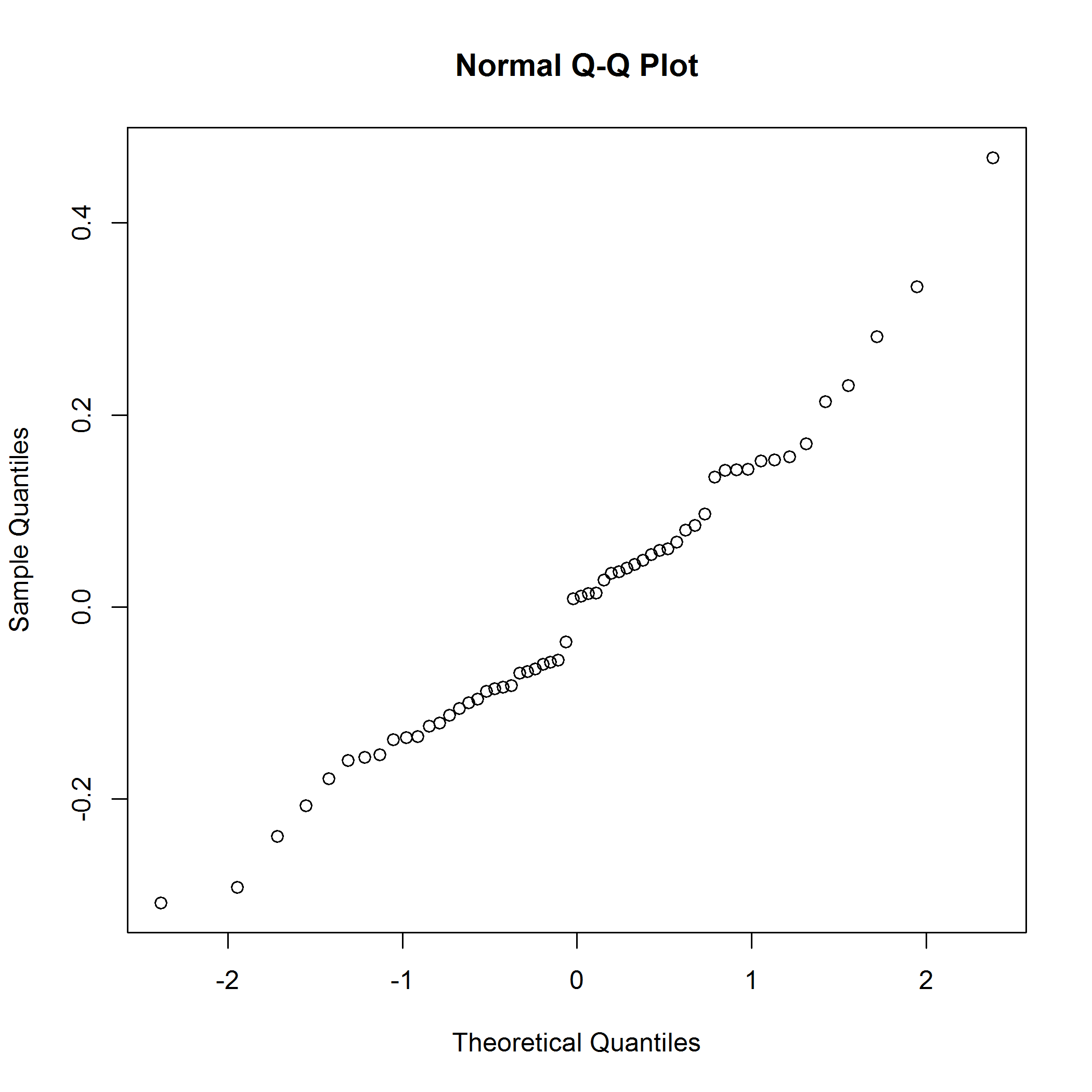
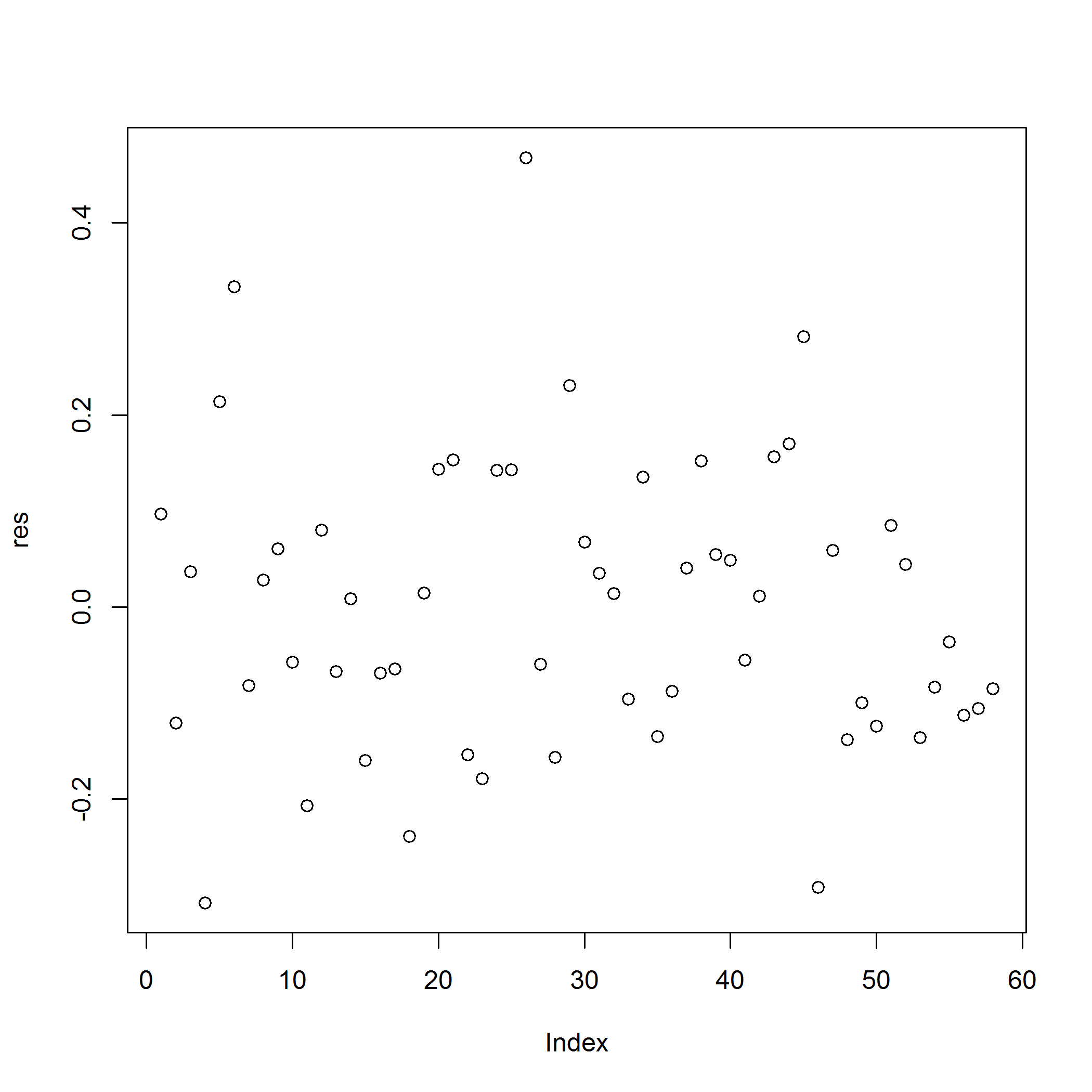
figures/lineplotNormalizedConcGSSG.png

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | group |
| (Intercept) | 0.83 | 0.07 | 12.19 | fixed |
| studyArmPlacebo | 0.08 | 0.09 | 0.90 | fixed |
| visitMonth3 | -0.07 | 0.08 | -0.90 | fixed |
| visitMonth12 | -0.04 | 0.08 | -0.52 | fixed |
| studyArmPlacebo:visitMonth3 | 0.15 | 0.11 | 1.32 | fixed |
| studyArmPlacebo:visitMonth12 | 0.13 | 0.11 | 1.12 | fixed |
| sd\_(Intercept).patientID | 0.11 | NA | NA | patientID |
| sd\_Observation.Residual | 0.17 | NA | NA | Residual |

## Linear mixed model fit by REML ['lmerMod']  
## Formula: normalizedConcGSSG ~ studyArm \* visitMonth + (1 | patientID)  
## Data: df  
##   
## REML criterion at convergence: -7.1  
##   
## Scaled residuals:   
## Min 1Q Median 3Q Max   
## -1.7631 -0.5947 0.0578 0.4788 2.6768   
##   
## Random effects:  
## Groups Name Variance Std.Dev.  
## patientID (Intercept) 0.01141 0.1068   
## Residual 0.03055 0.1748   
## Number of obs: 58, groups: patientID, 20  
##   
## Fixed effects:  
## Estimate Std. Error t value  
## (Intercept) 0.82904 0.06801 12.190  
## studyArmPlacebo 0.08238 0.09139 0.901  
## visitMonth3 -0.07445 0.08240 -0.904  
## visitMonth12 -0.04205 0.08073 -0.521  
## studyArmPlacebo:visitMonth3 0.15070 0.11443 1.317  
## studyArmPlacebo:visitMonth12 0.12535 0.11179 1.121  
##   
## Correlation of Fixed Effects:  
## (Intr) stdyAP vstMn3 vstM12 sAP:M3  
## stdyArmPlcb -0.739   
## visitMonth3 -0.606 0.451   
## visitMnth12 -0.637 0.485 0.510   
## stdyArmP:M3 0.437 -0.591 -0.720 -0.366   
## stdyArP:M12 0.458 -0.619 -0.369 -0.727 0.480

## $patientID

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | p.value |
| (Intercept) | 0.83 | 0.07 | 12.19 | 0.0000 |
| studyArmPlacebo | 0.08 | 0.09 | 0.90 | 0.3739 |
| visitMonth3 | -0.07 | 0.08 | -0.90 | 0.3728 |
| visitMonth12 | -0.04 | 0.08 | -0.52 | 0.6059 |
| studyArmPlacebo:visitMonth3 | 0.15 | 0.11 | 1.32 | 0.1969 |
| studyArmPlacebo:visitMonth12 | 0.13 | 0.11 | 1.12 | 0.2703 |

## Linear mixed-effects model fit by REML  
## Data: df   
## AIC BIC logLik  
## 12.94192 32.45436 3.529039  
##   
## Random effects:  
## Formula: ~1 | patientID  
## (Intercept)  
## StdDev: 0.1068296  
##   
## Formula: ~1 | visitMonth %in% patientID  
## (Intercept) Residual  
## StdDev: 0.1631421 0.0627639  
##   
## Correlation Structure: AR(1)  
## Formula: ~1 | patientID/visitMonth   
## Parameter estimate(s):  
## Phi   
## 0   
## Fixed effects: normalizedConcGSSG ~ studyArm \* visitMonth   
## Value Std.Error DF t-value p-value  
## (Intercept) 0.8290378 0.06800693 33 12.190490 0.0000  
## studyArmPlacebo 0.0823790 0.09139115 33 0.901389 0.3739  
## visitMonth3 -0.0744497 0.08240097 33 -0.903505 0.3728  
## visitMonth12 -0.0420507 0.08072781 33 -0.520895 0.6059  
## studyArmPlacebo:visitMonth3 0.1507045 0.11443206 33 1.316978 0.1969  
## studyArmPlacebo:visitMonth12 0.1253526 0.11179203 33 1.121301 0.2703  
## Correlation:   
## (Intr) stdyAP vstMn3 vstM12 sAP:M3  
## studyArmPlacebo -0.739   
## visitMonth3 -0.606 0.451   
## visitMonth12 -0.637 0.485 0.510   
## studyArmPlacebo:visitMonth3 0.437 -0.591 -0.720 -0.366   
## studyArmPlacebo:visitMonth12 0.458 -0.619 -0.369 -0.727 0.480  
##   
## Standardized Within-Group Residuals:  
## Min Q1 Med Q3 Max   
## -0.63307395 -0.21354564 0.02075229 0.17191976 0.96112891   
##   
## Number of Observations: 58  
## Number of Groups:   
## patientID visitMonth %in% patientID   
## 20 58

