Multiple Linear Regression of Human Beta-Carotene Levels

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Background

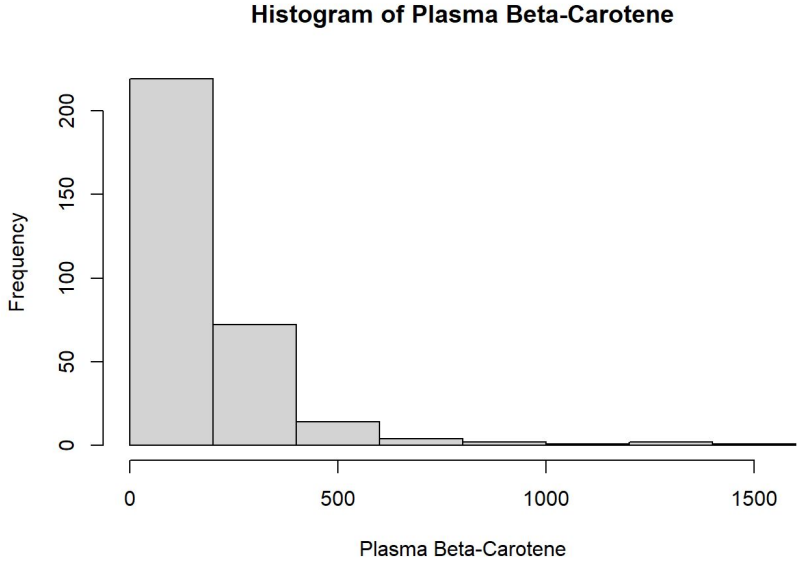
- Prior literature has shown a negative relationship between beta-carotene levels and risk of cancer
- Studying the determinants of beta-carotene levels can inform avenues for cancer prevention
- Past studies have shown several variables to be good predictors (carotene intake, gender, etc.)
- This study was designed to evaluate the effect of multiple predictors on both beta-carotene and retinol
- Our analysis focuses on beta-carotene: studies have shown this to have a stronger association with cancer - more important to study predictors of beta-carotene
- Goal will be to derive a model that is good at prediction and accurately quantifies relationships

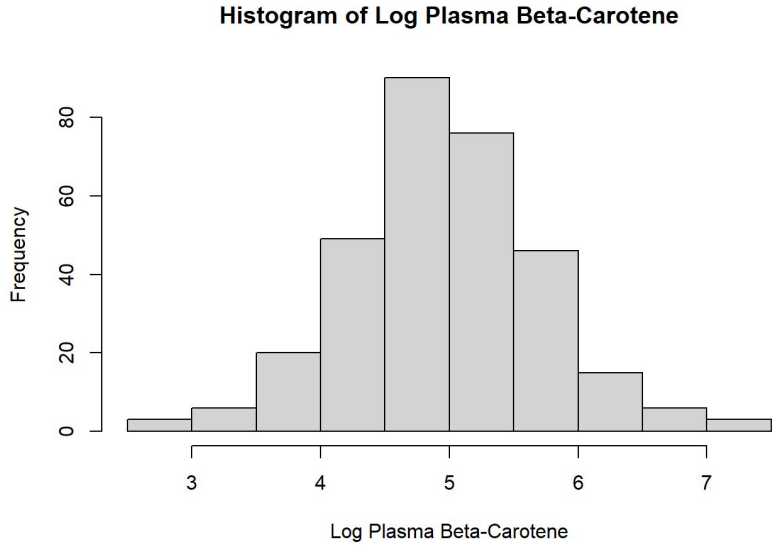
Dataset

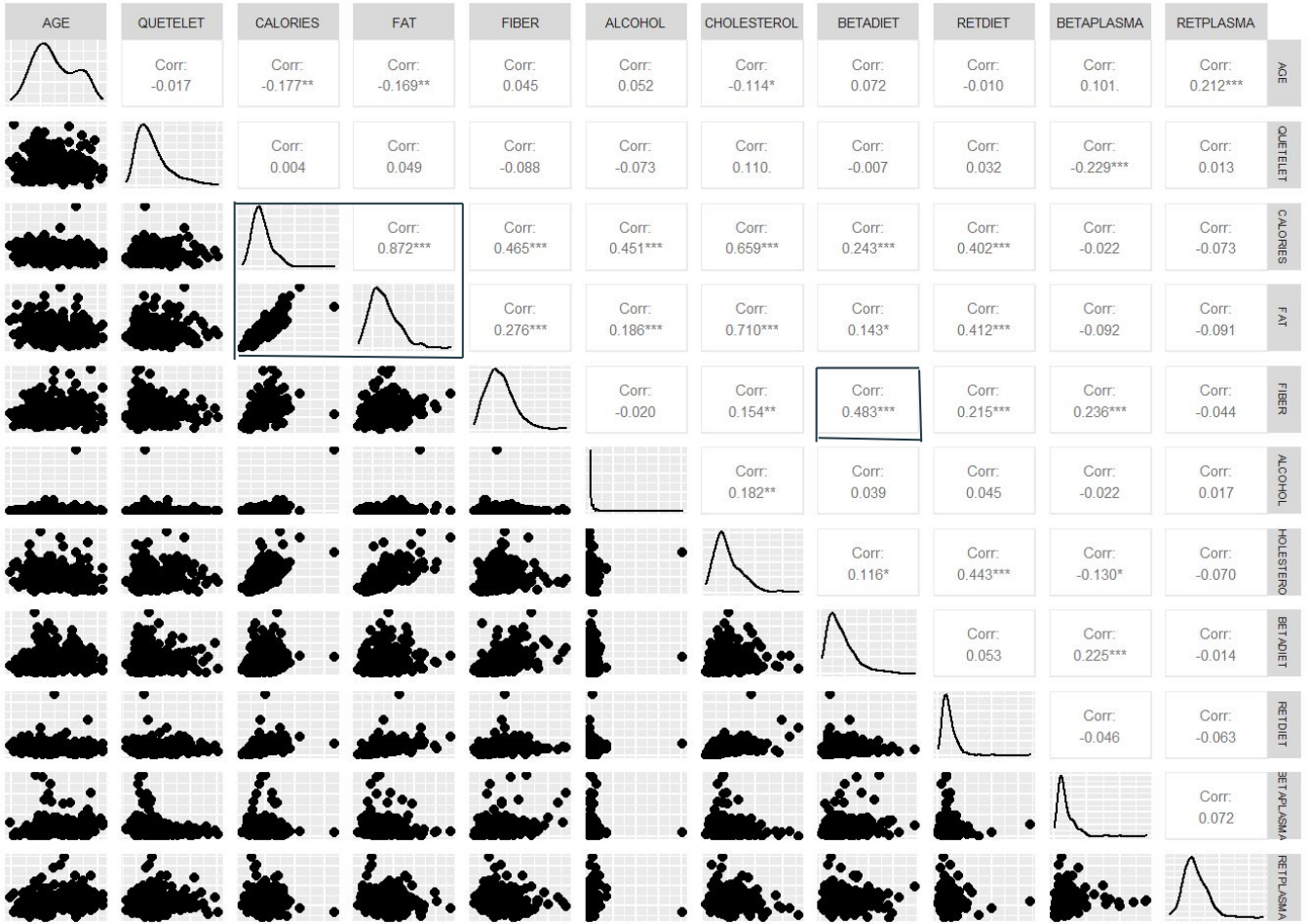
- 315 patients who had an elective surgical procedure during a three-year period to biopsy or remove a lesion of the lung, colon, breast, skin, ovary or uterus that was found to be non-cancerous.
  - Important that these patients did not have cancer: so beta-carotene levels are more representative of population
- Variables to note:
  - Quetelet - weight / height^2 (body mass index) - approximate measure of body fat
  - Beta-carotene levels (ng/ml): removed one observation where value was zero (measurement error)
  - Beta-carotene intake (mcg per day): approximate measure, based on frequency of consumption of certain foods
  - Three levels of smoking status, three levels of vitamin use (shown later)
- Mainly females, people of all ages (19 - 83)

Exploratory Data Analysis: Response Variable

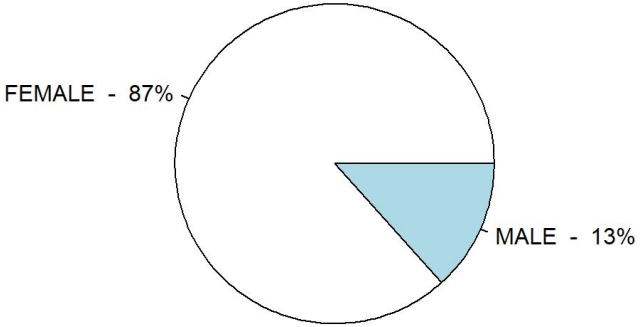
- Response variable is very right-skewed, so log transformation makes sense for normal error model



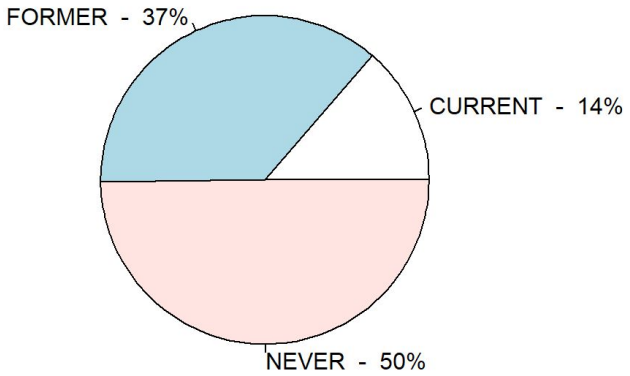



AGE
QUETELET
CALORIES
FAT
FIBER
ALCOHOL
CHOLESTEROL
BETADIET
RETDIET
BETAPLASMA
RETPLASMA
Corr: -0.017
Corr: -0.177**
Corr: -0.169**
Corr: 0.045
Corr: 0.052
Corr: -0.114*
Corr: 0.072
Corr: -0.010
Corr: 0.101.
Corr: 0.212***
Corr: 0.004
Corr: 0.049
Corr: -0.088
Corr: -0.073
Corr: 0.110.
Corr: -0.007
Corr: 0.032
Corr: -0.229***
Corr: 0.013
Corr: 0.872***
Corr: 0.465***
Corr: 0.451***
Corr: 0.659***
Corr: 0.243***
Corr: 0.402***
Corr: -0.022
Corr: -0.073
Corr: 0.276***
Corr: 0.186***
Corr: 0.710***
Corr: 0.143*
Corr: 0.412***
Corr: -0.092
Corr: -0.091
Corr: -0.020
Corr: 0.154**
Corr: 0.483***
Corr: 0.215***
Corr: 0.236***
Corr: -0.044
Corr: 0.182**
Corr: 0.039
Corr: 0.045
Corr: -0.022
Corr: 0.017
Corr: 0.116*
Corr: 0.443***
Corr: -0.130*
Corr: -0.070
Corr: 0.053
Corr: 0.225***
Corr: -0.014
Corr: -0.046
Corr: -0.063
Corr: 0.072

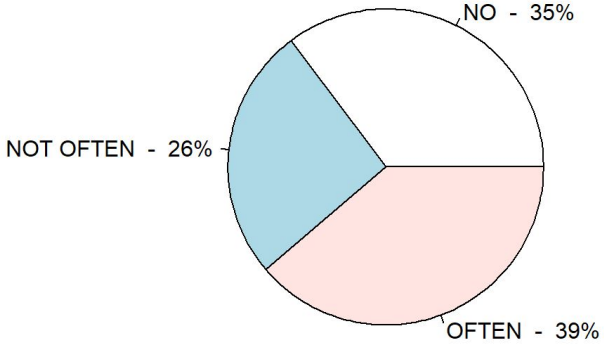
SEX: pie chart

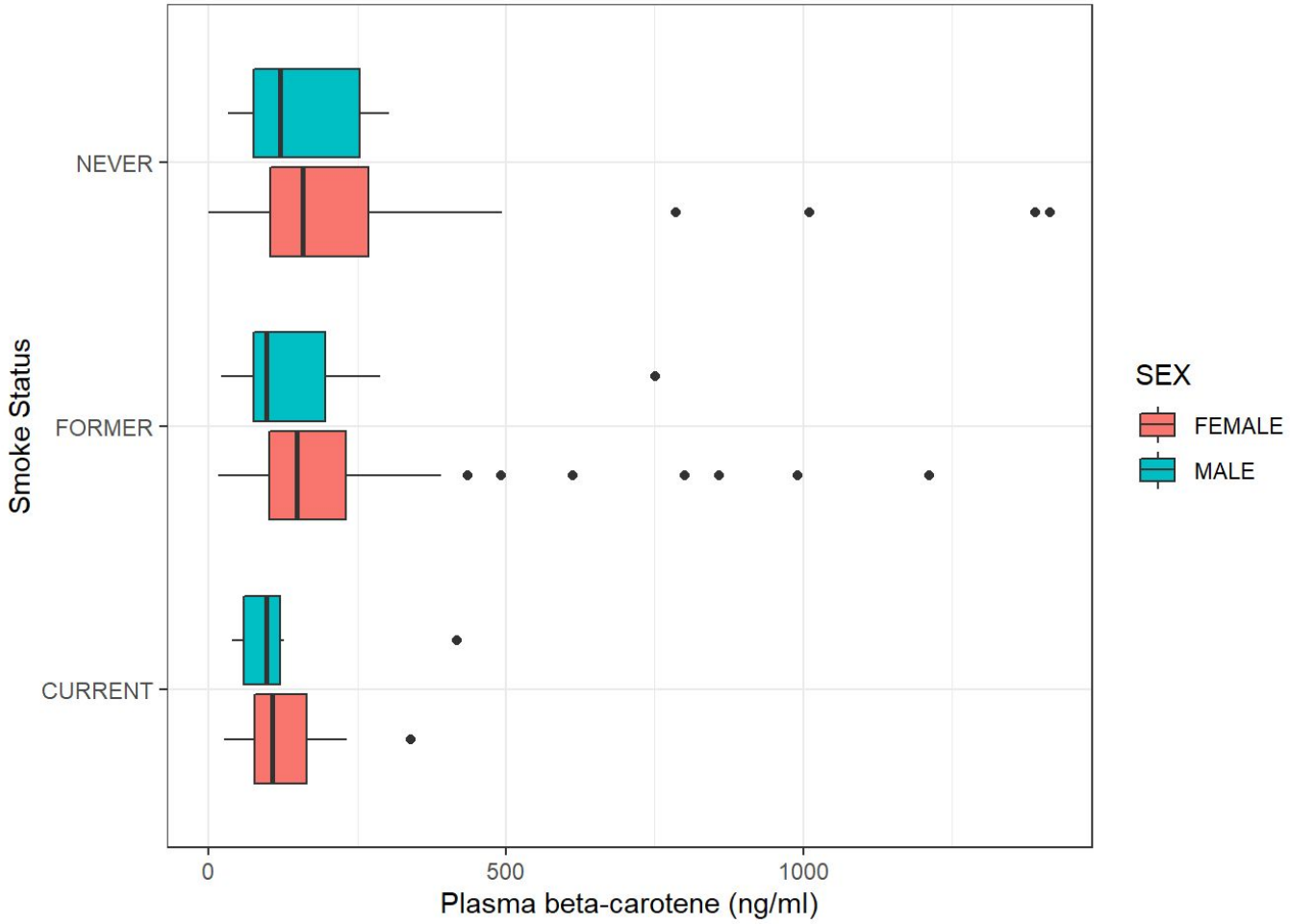


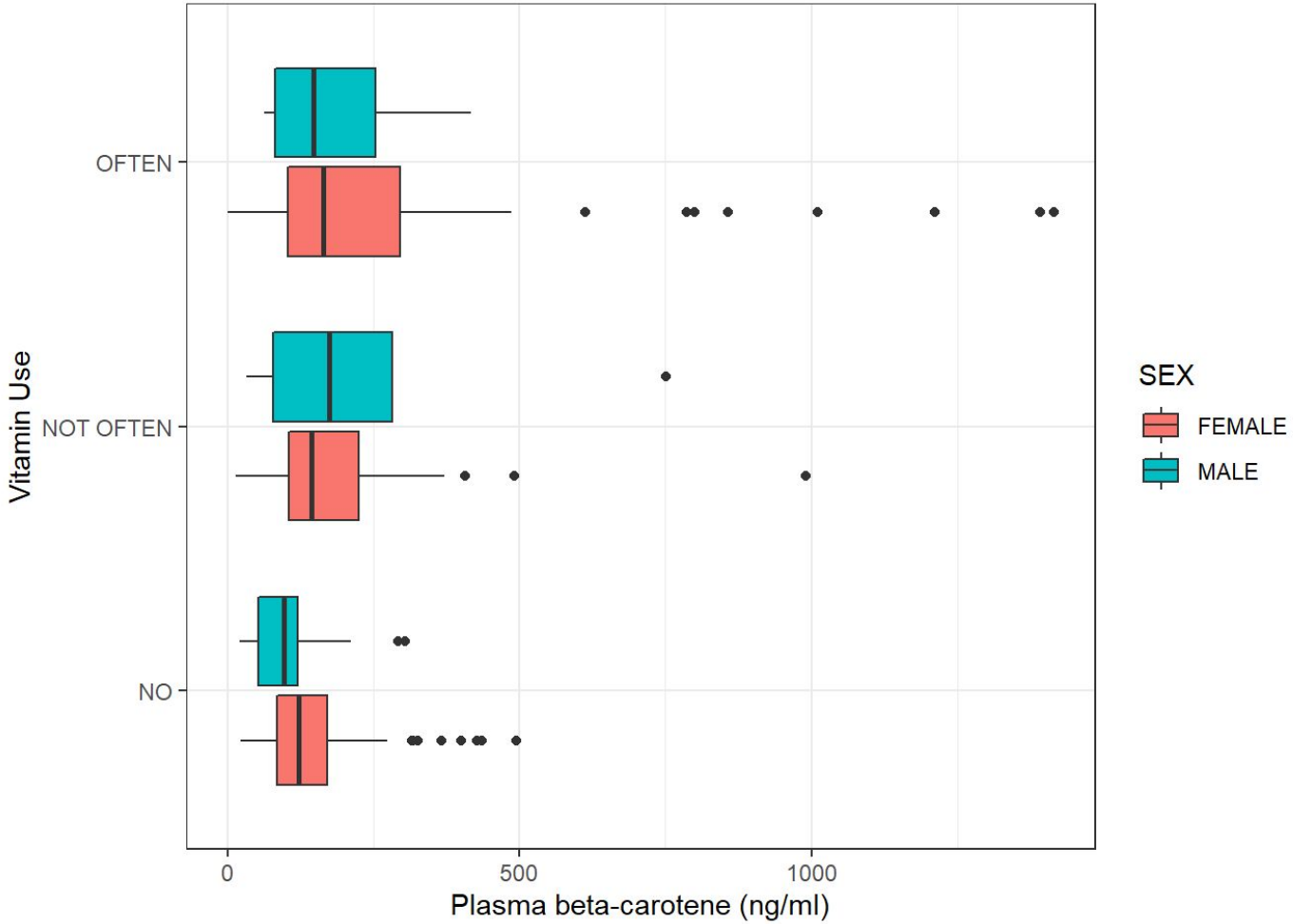
SMOKSTAT: pie chart



VITUSE: pie chart

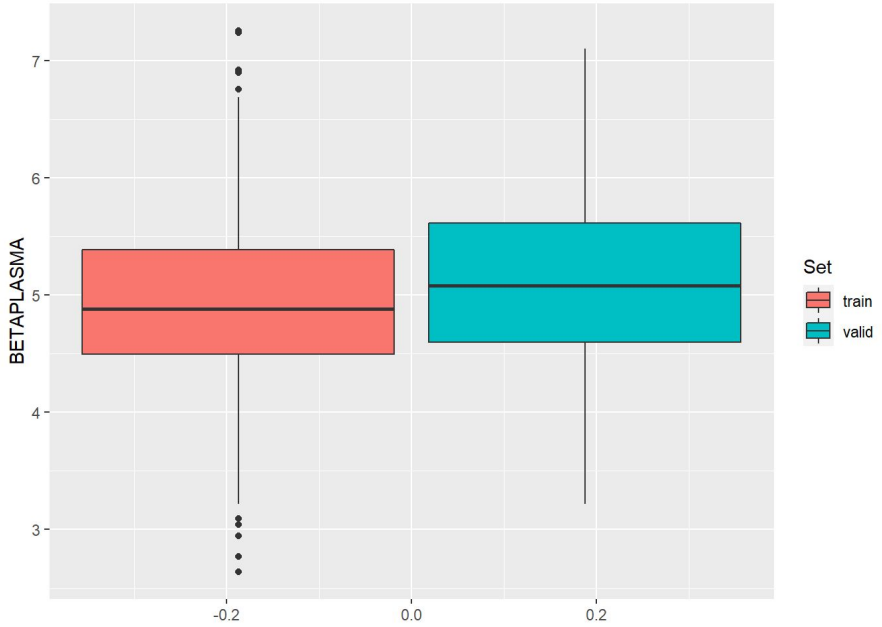



NEVER
FORMER
CURRENT
Smoke Status
0
500
1000
Plasma beta-carotene (ng/ml)
SEX
FEMALE
MALE


Vitamin Use
OFTEN
NOT OFTEN
NO
0
500
1000
Plasma beta-carotene (ng/ml)
SEX
FEMALE
MALE

Splitting Data into Training and Validation

- Split dataset 80/20
- Training dataset 250
- ~11 possible explanatory variables (14 possible coefficients)
- Many more possible interactions
- Since we have a smaller sample, we choose to use a “larger” training set
- Confirm that training and validation are similarly distributed



Model Selection Procedure

- Remove retinol variables from consideration
- Stepwise Regression - additive model
- Consider adding/removing additional variables based on literature and exploratory analysis
- Stepwise Regression - interaction model
- Decide if these interactions and/or other interactions should be added
- Land on final model and execute model diagnostics.

Stepwise Regression: Additive Model

- Choose to use AIC as criterion
- Variables consistent with literature
- Others worth considering for model

```
BETAPLASMA ~ QUETELET + FIBER + CALORIES + VITUSE + FAT + BETADIET
```

	Step	Df	Deviance	Resid. Df	Resid. Dev	AIC
1				249	141.4577	-140.3651
2	+ QUETELET	1	11.2686428	248	130.1891	-159.1183
3	+ FIBER	1	7.8677107	247	122.3213	-172.7023
4	+ CALORIES	1	5.6565390	246	116.6648	-182.5390
5	+ VITUSE	2	5.0691770	244	111.5956	-189.6448
6	+ FAT	1	1.4723217	243	110.1233	-190.9651
7	+ BETADIET	1	0.9336995	242	109.1896	-191.0938

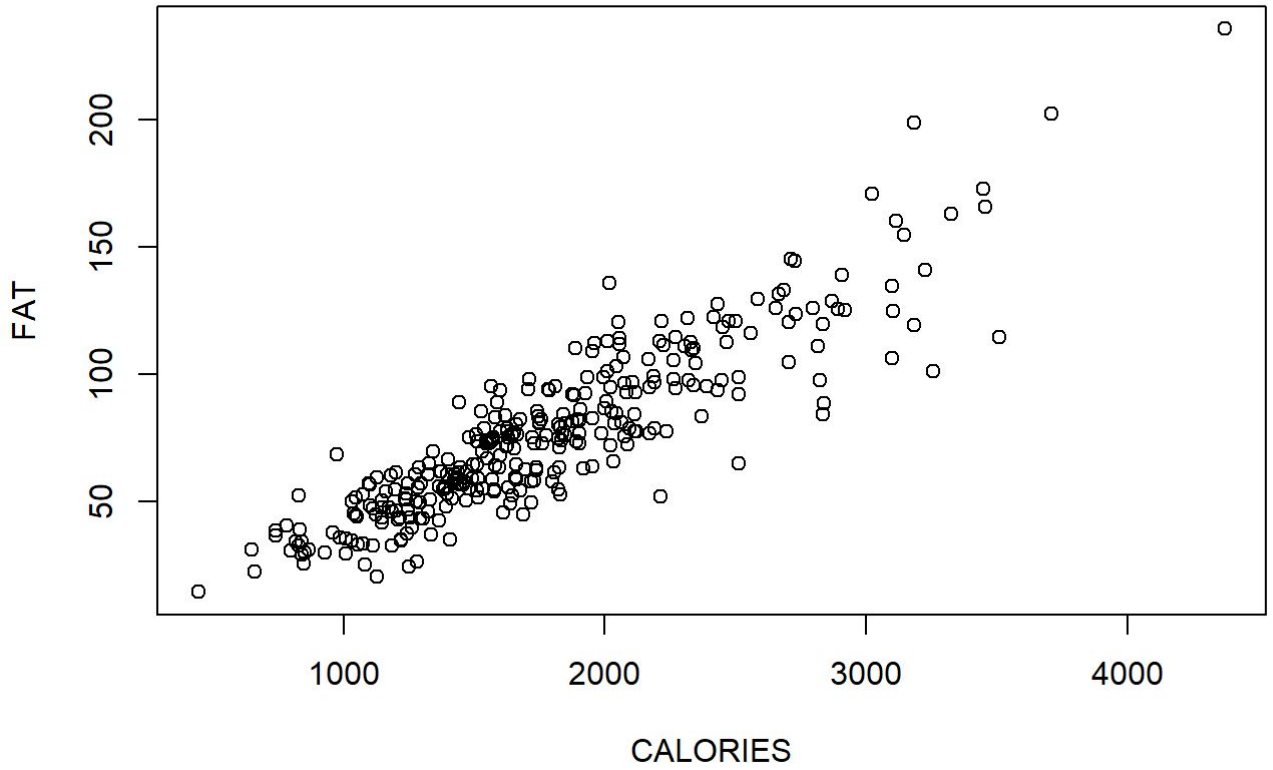
Alter Stepwise Model Based on Other Evidence

- Keep beta-carotene intake, quetelet, vitamin use: literature
- Also keep calories and fiber, very significant
- Stepwise not necessarily best model
    - May only be local optimum, and makes decisions based on very marginal differences in AIC
- Add gender and smoking: based on literature, exploratory analysis, and best subset selection
- Remove fat, high collinearity with calories, stepwise chose calories first
- Preferred additive model: initial results

```
Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)      5.301e+00  2.499e-01  21.209  < 2e-16 ***
QUETELET        -3.061e-02  6.989e-03  -4.380 1.78e-05 ***
FIBER            3.793e-02  1.054e-02   3.599 0.000388 ***
CALORIES        -2.599e-04  8.783e-05  -2.959 0.003393 **
VITUSENOT OFTEN  2.269e-01  1.126e-01   2.015 0.045054 *
VITUSEOFTEN      2.797e-01  1.030e-01   2.716 0.007080 **
BETADIET         4.764e-05  3.413e-05   1.396 0.164038
SEXMALE         -1.536e-01  1.443e-01  -1.064 0.288211
SMOKSTATFORMER   1.723e-01  1.354e-01   1.272 0.204597
SMOKSTATNEVER    2.113e-01  1.341e-01   1.576 0.116404
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.6734 on 240 degrees of freedom
Multiple R-squared:  0.2306, Adjusted R-squared:  0.2017
F-statistic: 7.992 on 9 and 240 DF,  p-value: 2.458e-10
```

Fat vs. Calories: Correlation Coefficient = 0.897



Stepwise Regression: Interaction Model

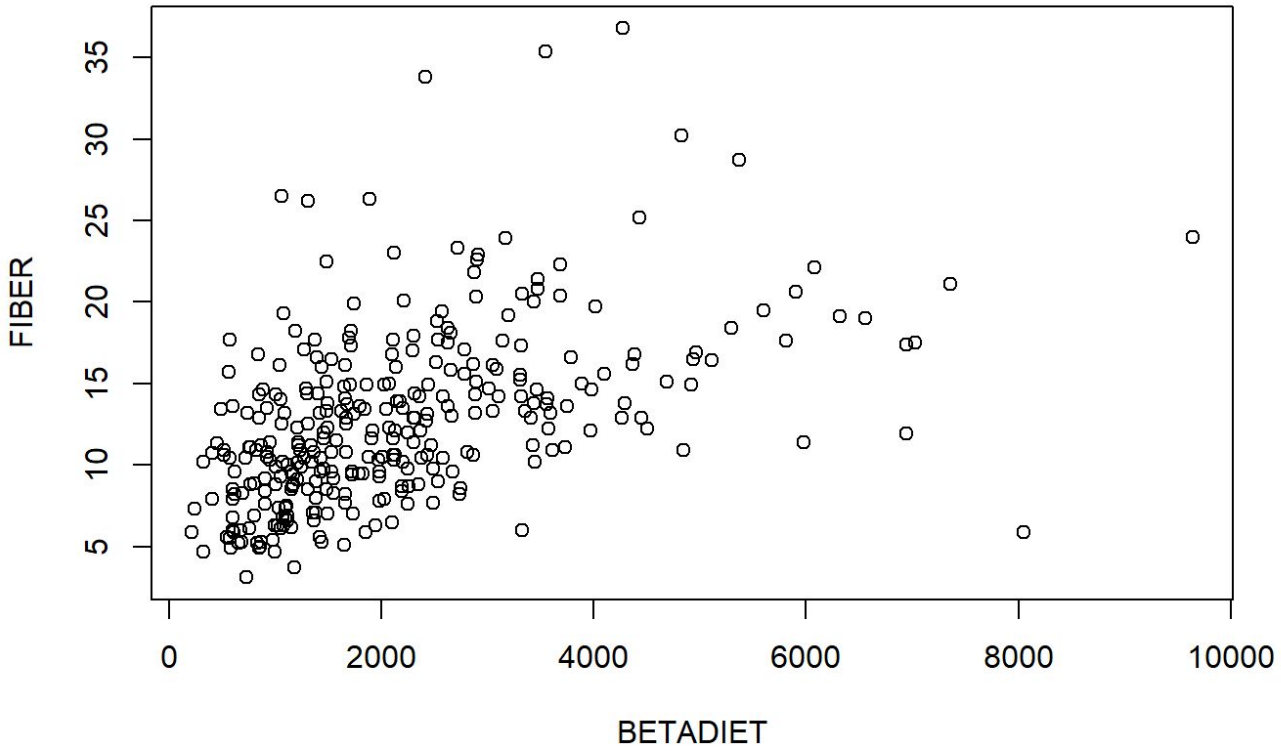
- Allow all two-way interactions
- Interaction between smoking status and vitamin use not justified by literature
- Interaction between sex and carotene intake could make sense, given past literature - propose adding to model
- Conduct further investigation on interaction between smoking status and fiber

```
Initial Model:
BETAPLASMA ~ QUETELET + FIBER + CALORIES + VITUSE + BETADIET +
    SEX + SMOKSTAT

Final Model:
BETAPLASMA ~ QUETELET + FIBER + CALORIES + VITUSE + BETADIET +
    SEX + SMOKSTAT + VITUSE:SMOKSTAT + BETADIET:SEX + FIBER:SMOKSTAT
```

	Step	Df	Deviance	Resid. Df	Resid. Dev	AIC
1				240	108.83739	-187.9015
2	+ SMOKSTAT:VITUSE	4	6.003025	236	102.83436	-194.0853
3	+ SEX:BETADIET	1	2.752223	235	100.08214	-198.8674
4	+ SMOKSTAT:FIBER	2	2.281181	233	97.80096	-200.6316

Fiber vs. Beta-Carotene Intake: Correlation Coefficient = 0.485



Final Model With Interactions

- Confirms findings from prior literature
  - Weight/height^2
  - Vitamin use
- Confirms interaction between carotene intake and smoking status
- Suggests interaction effect between gender and carotene intake
- Calories: important for model
- Effect of beta-carotene intake confounded by fiber and interaction

```
lm(formula = BETAPLASMA ~ QUETELET + FIBER + CALORIES + VITUSE +
    BETADIET + SEX + SMOKSTAT + BETADIET:SMOKSTAT + BETADIET:SEX,
    data = train)

Residuals:
     Min       1Q   Median       3Q      Max
-2.08246 -0.36923  0.00345  0.37187  1.94988

Coefficients:
                          Estimate Std. Error t value Pr(>|t|)
(Intercept)             5.473e+00  2.765e-01  19.791  < 2e-16 ***
QUETELET               -2.864e-02  6.914e-03  -4.143 4.77e-05 ***
FIBER                   3.907e-02  1.043e-02   3.746 0.000226 ***
CALORIES               -2.634e-04  8.699e-05  -3.028 0.002733 **
VITUSENOT OFTEN         2.044e-01  1.110e-01   1.842 0.066666 .
VITUSEOFTEN             2.725e-01  1.014e-01   2.686 0.007737 **
BETADIET               -8.357e-05  9.113e-05  -0.917 0.360024
SEXMALE                 5.309e-01  3.016e-01   1.760 0.079674 .
SMOKSTATFORMER         -8.318e-02  2.267e-01  -0.367 0.713946
SMOKSTATNEVER          -1.341e-01  2.192e-01  -0.612 0.541339
BETADIET:SMOKSTATFORMER 1.432e-04  1.009e-04   1.420 0.156983
BETADIET:SMOKSTATNEVER  1.847e-04  9.789e-05   1.887 0.060408 .
BETADIET:SEXMALE       -3.263e-04  1.295e-04  -2.520 0.012405 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.6621 on 237 degrees of freedom
Multiple R-squared:  0.2655, Adjusted R-squared:  0.2283
F-statistic: 7.138 on 12 and 237 DF,  p-value: 4.339e-11
```

Validation

Internal Validation:

- Press P measures predictive ability of model using LOOCV
- Press P = **119.68**, SSE = **103.90**

External Validation:

- Mean Squared Prediction Error measures predictive ability of model using hold out data (20% of initial dataset, 63 observations)
- MSPE = **0.445**, SSE (training data) / 250 = **0.416**

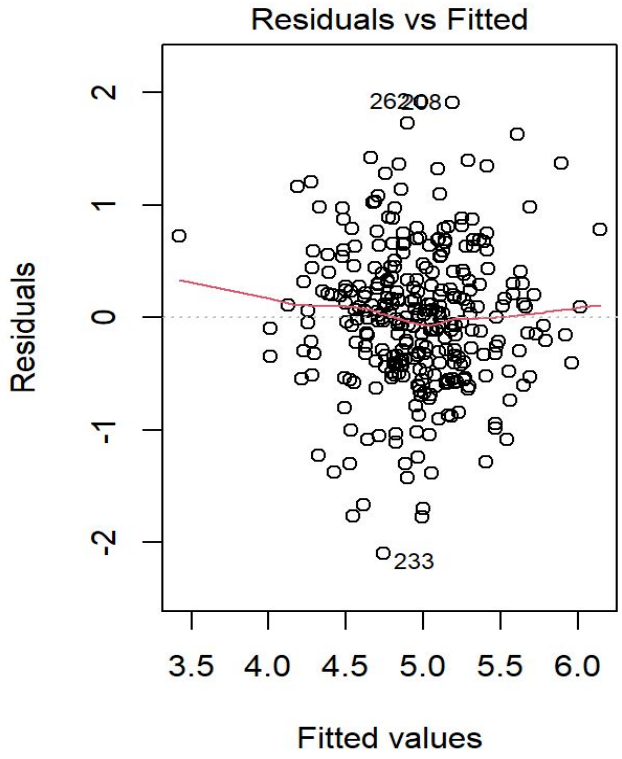
Refit Model on Full Dataset

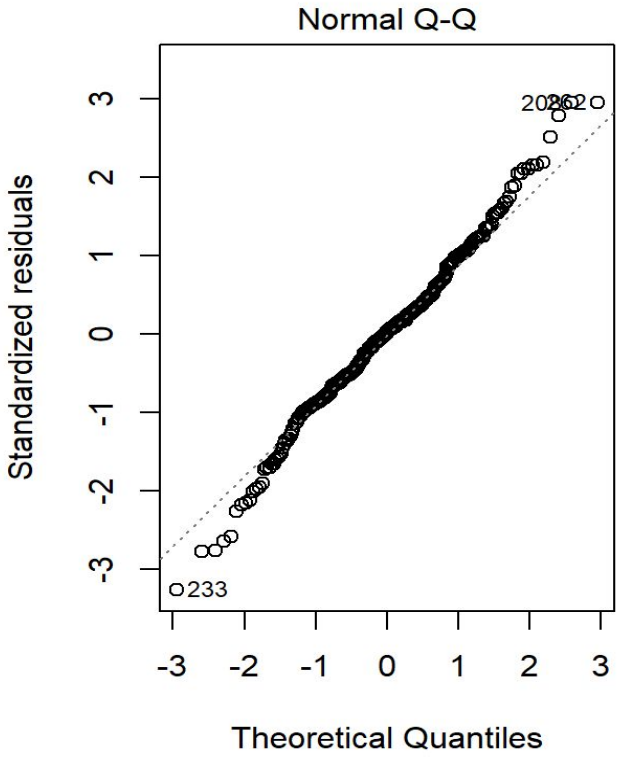
- Larger sample size decreases standard errors
- Magnitude and sign of coefficient estimates pretty similar to model fit on training data
- Some small differences
  - Vitamin use more significant
  - Gender less of an effect
  - Smoking status and carotene intake interaction more significant

```
Coefficients:
                           Estimate Std. Error t value Pr(>|t|)
(Intercept)               5.564e+00  2.529e-01  22.003  < 2e-16 ***
QUETELET                 -3.076e-02  6.339e-03  -4.852 1.96e-06 ***
FIBER                     3.383e-02  9.306e-03   3.636 0.000326 ***
CALORIES                 -2.322e-04  7.310e-05  -3.177 0.001645 **
VITUSENOT OFTEN           2.281e-01  9.792e-02   2.329 0.020514 *
VITUSEOFTEN               2.922e-01  8.924e-02   3.274 0.001185 **
BETADIET                 -1.451e-04  8.365e-05  -1.734 0.083913 .
SEXMALE                   1.518e-01  2.488e-01   0.610 0.542338
SMOKSTATFORMER           -1.402e-01  2.055e-01  -0.682 0.495571
SMOKSTATNEVER            -9.499e-02  2.016e-01  -0.471 0.637854
BETADIET:SMOKSTATFORMER   2.162e-04  9.033e-05   2.393 0.017325 *
BETADIET:SMOKSTATNEVER    2.343e-04  9.005e-05   2.602 0.009726 **
BETADIET:SEXMALE         -1.384e-04  1.006e-04  -1.377 0.169657
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.6565 on 300 degrees of freedom
Multiple R-squared:  0.2604, Adjusted R-squared:  0.2308
F-statistic: 8.803 on 12 and 300 DF,  p-value: 2.052e-14
```

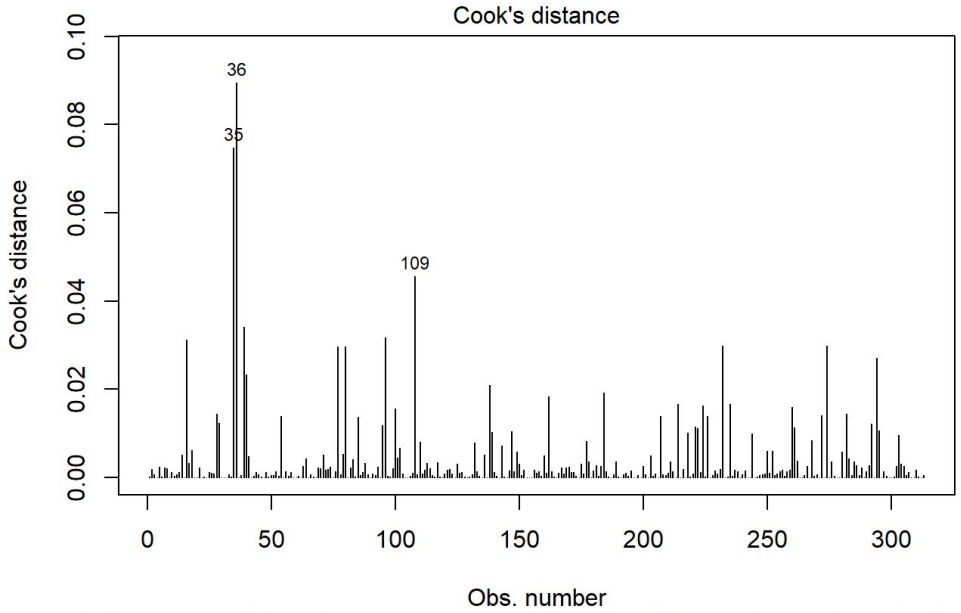
Model Diagnostics





Model Diagnostics: Cont'd

- Several influential cases, but most influential are obs 35 and 36
- 35 has very low carotene intake but high carotene levels
- 36 has very high carotene intake but low carotene levels



Discussion

Findings

- Negatively associated with BMI, smoking, and calorie intake
- Positively associated with taking vitamins, fiber intake, and carotene intake (assuming not smoking)

Limitations

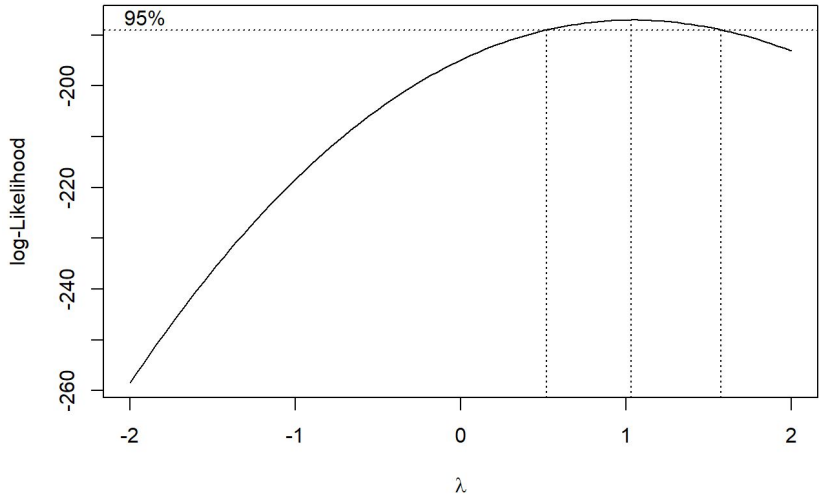
- Inaccuracy of beta carotene intake measurements - cause influential cases and may reduce accuracy of coefficients
- Unclear what vitamins are being taken
- Multicollinearity between fat and calories as well as between fiber and carotene intake
- Small sample size, so coefficient estimates may be sensitive to sampling variability
    - e.g. gender no longer statistically significant after running full model (compared to model ran on training data)

Conclusion

- External validity seems decent, diverse age, BMI, and habits
- Results are consistent with literature
- This can inform action: what can patients do to reduce cancer risk
- This analysis cannot conclude causation, just association
- Predict carotene without directly measuring it (which could be more tedious/costly)
    - Instead, can predict beta-carotene levels using more accessible information (mass and height, daily calorie intake, etc.)
- Further areas of research
    - Scientific mechanism that some of these factors affect carotene levels
    - Smoking, calories vs. fat, fiber, vitamins
    - Larger studies

Fit Preliminary Model

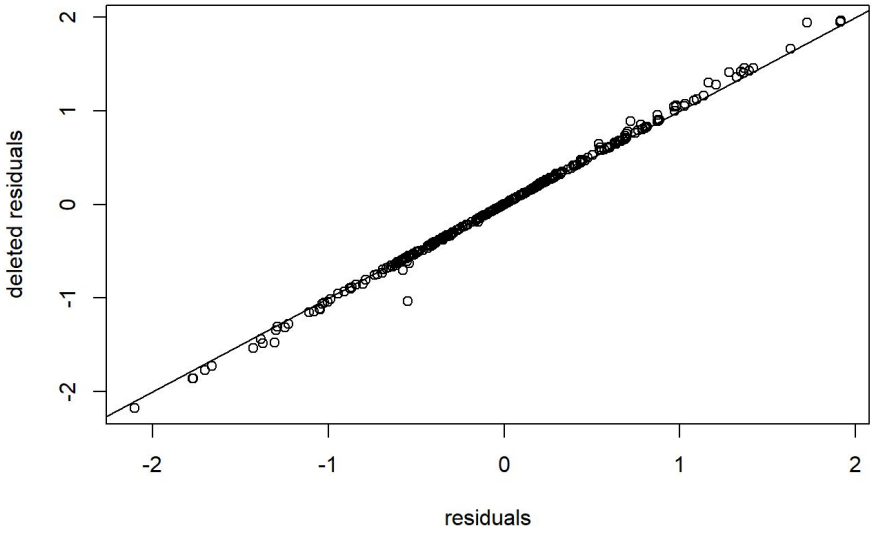
- All possible variables except for retinol levels and retinol intake, don't want to confound effects

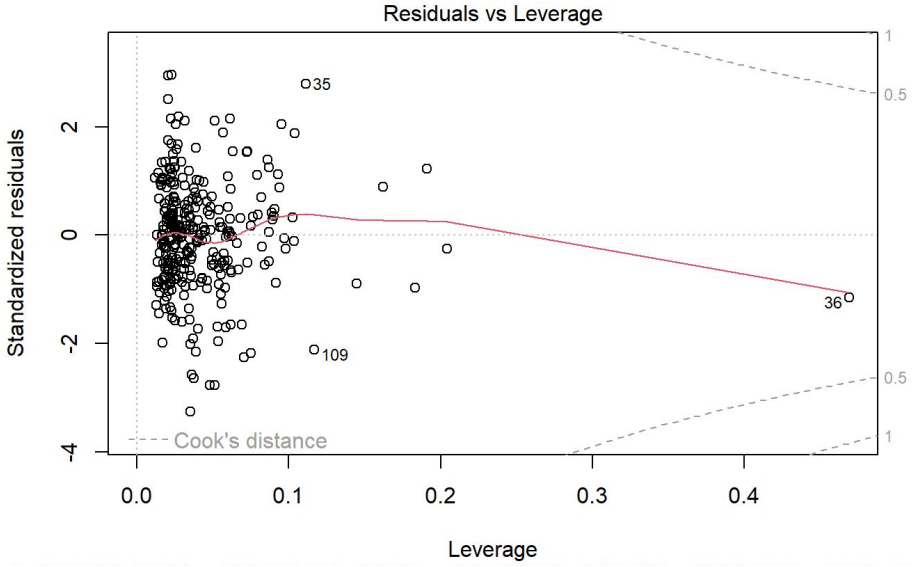


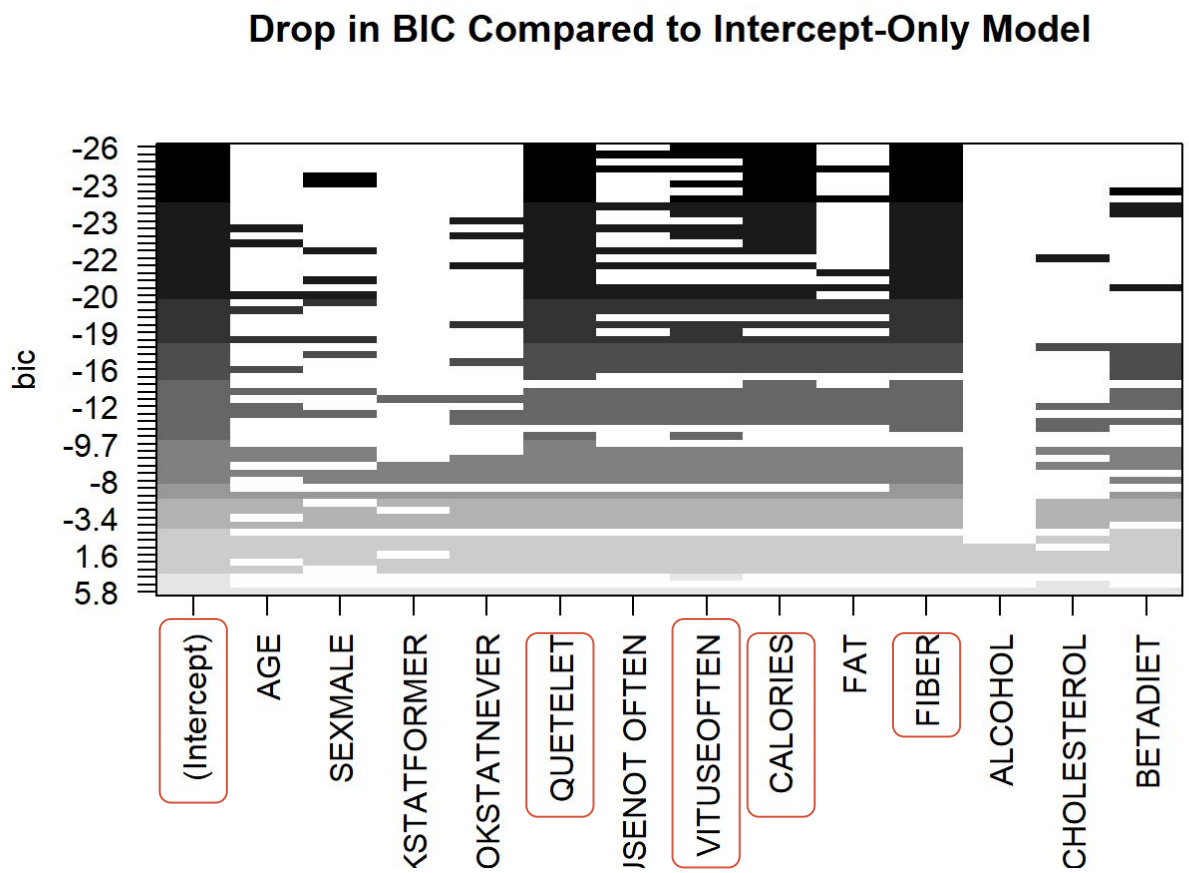

```
Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)    5.102e+00  3.012e-01  16.939  < 2e-16 ***
AGE            3.920e-03  3.341e-03   1.174 0.241772
SEXMALE       -1.889e-01  1.565e-01  -1.207 0.228522
SMOKSTATFORMER 1.522e-01  1.362e-01   1.117 0.264955
SMOKSTATNEVER  1.976e-01  1.345e-01   1.469 0.143111
QUETELET      -2.977e-02  7.134e-03  -4.173 4.23e-05 ***
VITUSENOT OFTEN 2.640e-01 1.133e-01   2.331 0.020610 *
VITUSEOFTEN    3.116e-01  1.056e-01   2.951 0.003488 **
CALORIES      -5.628e-04  2.520e-04  -2.233 0.026468 *
FAT            6.720e-03  3.811e-03   1.763 0.079181 .
FIBER          4.605e-02  1.316e-02   3.499 0.000558 ***
ALCOHOL        5.192e-03  9.955e-03   0.522 0.602470
CHOLESTEROL   -4.441e-04  4.979e-04  -0.892 0.373386
BETADIET       4.312e-05  3.449e-05   1.250 0.212416
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.671 on 236 degrees of freedom
Multiple R-squared:  0.2489, Adjusted R-squared:  0.2075
F-statistic: 6.015 on 13 and 236 DF,  p-value: 1.193e-09
```

Model Diagnostics: Cont'd






Drop in BIC Compared to Intercept-Only Model
bic
-26
-23
-23
-22
-20
-19
-16
-12
-9.7
-8
-3.4
1.6
5.8
(Intercept)
AGE
SEXMALE
KSTATFORMER
OKSTATNEVER
QUETELET
ISENOT OFTEN
VITUSEOFTEN
CALORIES
FAT
FIBER
ALCOHOL
CHOLESTEROL
BETADIET

EDA: Beta-Carotene vs. Retinol

- Not very correlated, so our analysis does not suggest much about predictors of retinol