

# EDA: Oxidative Stress, Inflammation, and Glucocorticoid Stress

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## Project Description

This report is part of a straightforward project intended to determine whether immune and stress responses cause/result in/associate with oxidative stress in a wild orangutan population. Some or all of these findings may be merged with the `tuanan_dietary_restriction_oxidative_stress_inflammation_cortisol` project focusing on DR. However, I want to work out this biomarker-only part first, and then relate them to diet, and see if the two stories should be merged or if the amount of information is too substantial to put them together.

The question is meta-analyzed in birds in Costantini and Moller. I will be focusing on oxidative stress (acronym: OS) as a response, with immune responses (WBC, neopterin, cytokines) and stress responses (cortisol) as potential drivers of increased oxidative stress. As part of this, I would want to test whether there are age-sex differences in oxidative stress, and if I can, try to detect seasonal trends perhaps using the GAMM approach of polansky and robbins 2013 or by one of Gavin Simpson's blogposts.

I may want to include the creatinine-residual data from caitlin, as there are myogenic and myoanabolic links between oxidative stress and lean muscle mass. ROS can stimulate expression and activity of skeletal muscle protein degradation pathways (muscle wasting).

Co-Authors: Erin R. Vogel, Rebecca S.A. Brittain, Tim Bransford (? cortisol), Alysse Moldawer (? cytokines), Lync Moldawer (? cytokines), Sri Suci Utami Atmoko, Caitlin S.A. O'Connell (ran a lot of neopterin).

How do orang neopterin levels compare to neopterin in other NHPs? - Probably answered in Liz's dissertation.

## Summary of EDA Findings

Results focus on standard inclusion criteria (first morning void, stored on ice in the field, CV below 15%, and specific gravity above 1.003) and for each biomarker, outliers are removed if they are more than 3 standard deviations away from the mean. Additionally, cortisol values are restricted to below 250,000.

For fitting mixed models, the following distributions were determined to be best:

- 8-OHdG - log-normal
- TAC - Gamma
- Neopterin - log-normal
- Cortisol - Gamma
- ELBM - normal

For covariation between biomarkers and age sex class, truncated classes were chosen. All infants and juveniles were grouped together, all adult females were grouped, and adult males were separated into flanged and unflanged.

Table 1: Table 1a: All assayed samples without any removed and no specific gravity correction applied

biomarker	non_na	minimum	maximum
8-OHdG	742	0.102	255.544
Cortisol	995	152.948	4405531.000
G-CSF	460	0.000	12864.000
IL-10	457	0.000	5383.000
IL-1Ra	452	0.000	507.750
IL-8/CXCL-8	459	0.000	2751.000
MCP-1	461	0.000	919.540
Neopterin	1165	1.560	14243.201
TNF_a	461	0.000	919.540
Total Antioxidant Capacity	554	0.000	36.538

- No apparent differences in 8-OHdG between age sex classes.
- No apparent differences in TAC between age sex classes.
- Some small differences in neopterin between age sex classes. Flanged Males exhibit higher inflammation.
- Seems flanged males might have higher cortisol levels but needs more exploration.

## Data Details

- dimensions
  - Number of samples per orangutan
  - number of samples per field worker
  - number of samples per fai tercile
  - number of samples per year
- biomarker data inclusion criteria
  - start with total number of samples with each measurement
  - remove poor CVs
  - remove low specific gravity
  - maybe remove 1.5 times IQR outliers, but definitely box plot before this step
  - non-first morning voids
  - contaminated with fecal matter
  - status not “ok”
- – Maria file from caitlin paper with individual IDs

## To Check

- orangutan names, make sure they’re correct
- double check age-classes
- if a cytokine is marked as zero, it was below the limit of detection
  - i say give it a random non-zero number that is within the LOD range

I still need to do something with the zero values for the cytokines, as they’re not really zero.

**How many biomarker values does each individual orangutan have?** I need to double check that the values in the database under Orangutan\_ID are indeed correct. Maria sent an excel file with a list of follow and sample numbers and the correct names and age\_sex classes. I should merge this in and use those instead of any values in the urine database because I am not sure they've been QC'd.

This table has been arranged by number of 8-OHdG samples in descending order. Total biomarker values indicates the sum of all assayed samples. I also only used samples that satisfied primary inclusion criteria: kept in thermos in field, SG not dilute, a first morning void, and the CV for the given assay sample was below 15%.

**How many assayed samples of each biomarker meet each inclusion criterion?** I want to look at each of my inclusion criterion to determine whether any lead to significant loss of sample numbers or change balance fo the data, ultimately reducing power. When I know that a given exclusion criterion reduces power, I can make a judgement call as to what to do.

The inclusion criteria are as follows:

1. Sample is not dilute (specific gravity > 1.003)
2. Sample assay was precise (CV is equal to or below 15%)
3. Sample is from a first morning void
4. Assay was determined to have run appropriately (Sample assay status is OK, not set to 'rerun')

Ok, I will calculate percentages for each of these variables, surround the percentages in parentheses and add a percent sign, then merge the pairs of columns together in the final product. Should be able to use column\_spec to add the parentheses and percentages.

After filtering out samples that were not stored in a cold thermos in the field, were not first morning voids, were too dilute, and had poor CVs, the following number of samples remained for each biomarker.

604, 446, 750, 543, 152, 149, 150, 164, 149, 174

**How many samples have a status of "ok"?** For this one I need to make sure all records have a value here. Otherwise some NAs may get removed. I have not yet created this table.

## Variation

**General Notes:** I am not yet sure how I want to deal with outliers. Many papers use the  $1.5 * IQR$  method; this is reasonable. The outliers themselves should be investigated to see if I can determine why they are outliers. If there is an error, I can remove them. If they are part of natural variation, I may keep or remove them. If they are removed but seem to be accurate values, I may want to provide supplementary information on the samples in a table providing any relevant information on them.

**8-OHdG Variation** There appears to be a few pretty large outliers in the >90 range. A natural log transformation creates a vaguely normal distribution with some pretty long tails on either end and the potential for a little bimodality around ln2.7. The skewness of the SG-corrected 8-OHdG is 4.4674973 and kurtosis is 33.2902414

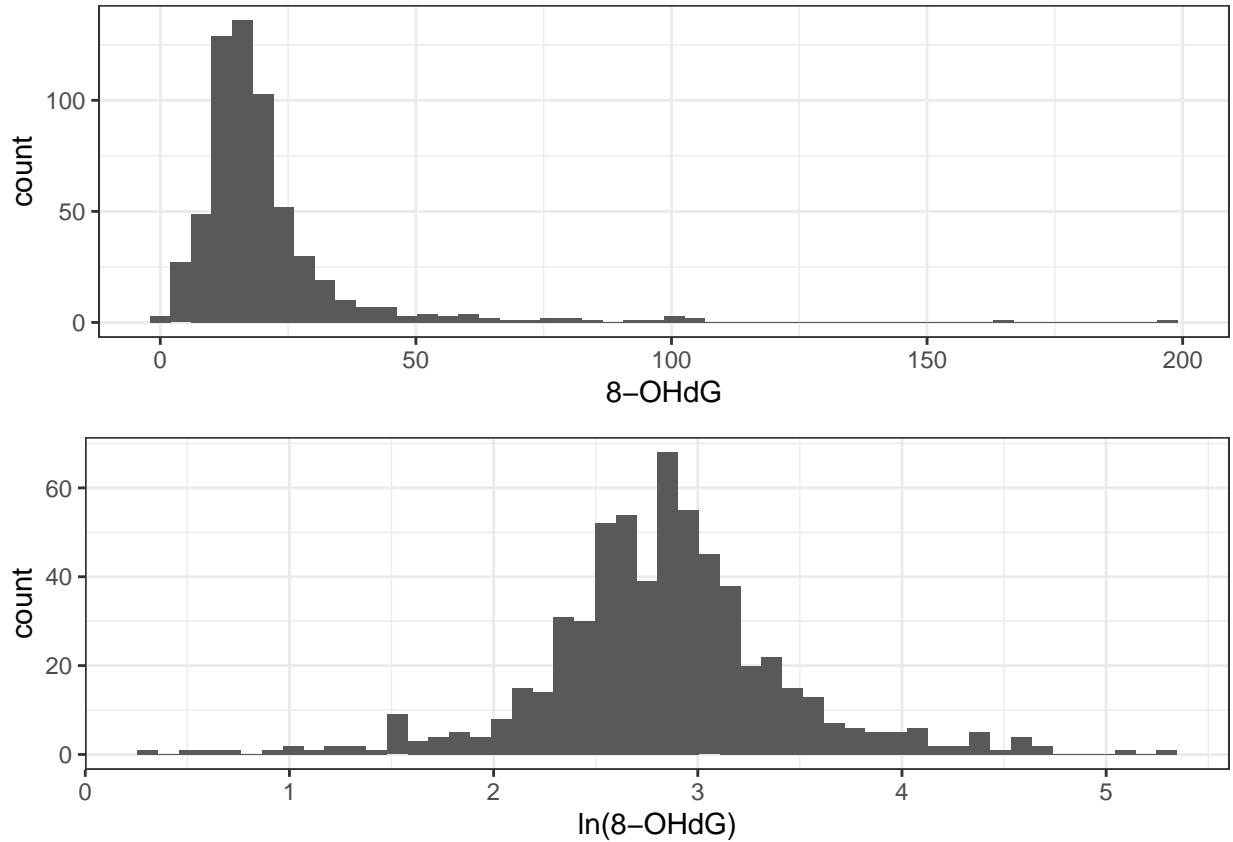
Table 2: Table 1c: All assayed samples by individual orangutan

Orangutan_ID	8-OHdG	Cortisol	G-CSF	IL-10	IL-1Ra	IL-8/CXCL-8	MCP-1	Neopterin	TN
Mindy	64	56	15	14	15	17	16	62	
Milo	49	37	11	10	9	10	8	60	
Juni	44	48	18	16	14	18	15	55	
Dayak	35	25	5	4	4	6	4	32	
Kerry	35	23	8	9	9	10	8	42	
Kondor	25	26	2	3	2	2	2	30	
Niko	25	29	6	6	5	4	4	31	
Tomi	25	25	5	5	5	5	3	28	
Jinak	24	31	12	11	10	11	11	23	
Inul	22	20	6	5	5	6	6	21	
Wodan	21	25	8	7	7	10	8	29	
Mawas	19	12	0	1	2	2	2	29	
Teju	13	9	0	0	1	0	1	11	
Dado	12	0	0	0	0	0	0	5	
Desy	12	5	4	4	4	4	4	16	
Henk	12	15	8	8	10	10	10	16	
Otto	12	15	4	4	5	5	5	14	
Sidony	12	10	13	13	13	13	13	15	
Ekko	11	6	1	1	1	1	1	17	
Gismo	11	5	1	1	1	1	1	17	
Helium	10	10	5	5	5	5	5	13	
Sugus	9	8	0	1	1	1	1	13	
Ted	9	7	0	0	0	0	0	10	
Jip	8	7	3	3	2	3	3	8	
Cinta	7	7	0	0	0	0	0	7	
Ipsy	6	6	1	1	1	1	1	9	
Pinky	6	12	0	0	0	0	0	16	
Unknown unflanged male	6	3	1	1	1	1	1	5	
Bobo	5	6	0	1	1	1	1	4	
Tina	5	7	0	0	0	0	0	10	
Vini	5	0	0	0	0	0	0	4	
Katmandun	4	4	3	3	4	3	3	2	
Sony	4	0	1	2	2	3	2	3	
Streisel	4	4	1	0	1	1	1	4	
Tarzan	4	5	3	3	3	3	2	5	
Charlie	3	2	0	0	0	0	0	3	
Kino	3	3	1	1	1	1	1	3	
Chaz	2	3	4	4	4	4	4	2	
Kiki	2	2	0	0	0	0	0	3	
Max	2	0	0	0	0	0	0	4	
Unknown flanged male	2	0	0	0	0	0	0	6	
Whisky	2	0	0	0	0	0	0	2	
Arthur	1	1	0	0	0	0	0	4	
Dolay	1	1	1	1	1	1	1	1	
Fugit	1	4	0	0	0	0	0	5	
Guapo	1	1	0	0	0	0	0	1	
Merkur	1	0	0	0	0	0	0	1	
MindyMawas	1	1	1	1	1	1	1	0	
Momo	1	2	0	0	0	0	0	1	
Preman flanged	1	0	0	0	0	0	0	0	
Tom	1	1	0	0	0	0	0	1	
Tuk-Tuk	1	1	4	0	0	0	0	1	
Unknown adolescent female	1	0	0	0	0	0	0	1	
Wilma	1	1	0	0	0	0	0	1	
Zola	1	1	0	0	0	0	0	1	

Table 3: Table 1d: The number of assayed samples by biomarker that meet each inclusion criterion

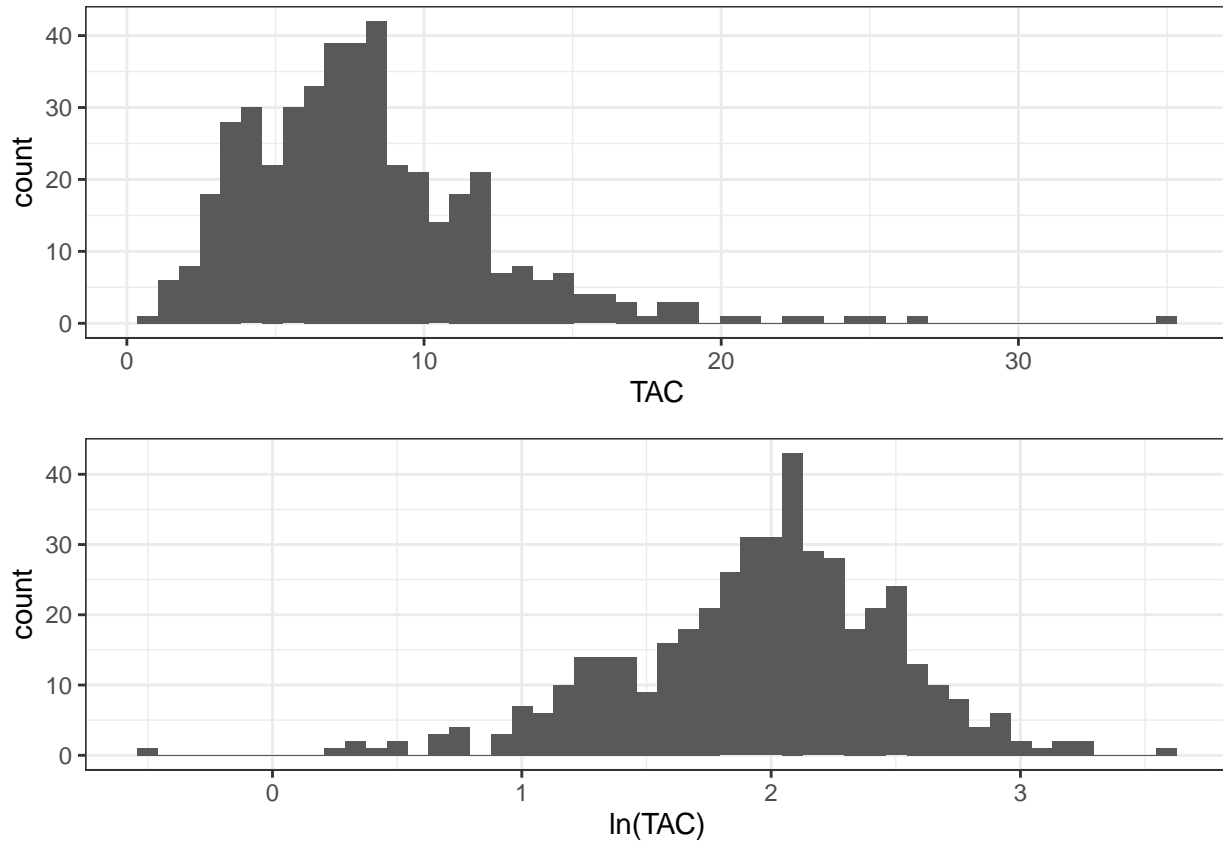
Biomarker	# of Total Assayed Samples	SG > 1.003	Stored in Thermos	First Morning Voids
8-OHdG	742	740 (99.73%)	715 (96.36%)	649 (87.47%)
Cortisol	995	932 (93.67%)	806 (81.01%)	685 (68.84%)
G-CSF	460	384 (83.48%)	291 (63.26%)	276 (60%)
IL-10	457	384 (84.03%)	287 (62.8%)	273 (59.74%)
IL-1Ra	452	376 (83.19%)	281 (62.17%)	266 (58.85%)
IL-8/CXCL-8	459	383 (83.44%)	289 (62.96%)	273 (59.48%)
MCP-1	461	385 (83.51%)	290 (62.91%)	275 (59.65%)
Neopterin	1165	1153 (98.97%)	1043 (89.53%)	846 (72.62%)
TNF_a	461	386 (83.73%)	291 (63.12%)	275 (59.65%)
Total Antioxidant Capacity	554	552 (99.64%)	537 (96.93%)	460 (83.03%)

The skewness of the natural logged 8-OHdG is -0.0017255 and the kurtosis is 5.4216779. So there is a considerable reduction in both skew and kurtosis after a natural log transformation, but the distribution of these data should be explored further. Is there a reason for that near-bimodality? Age-sex class?

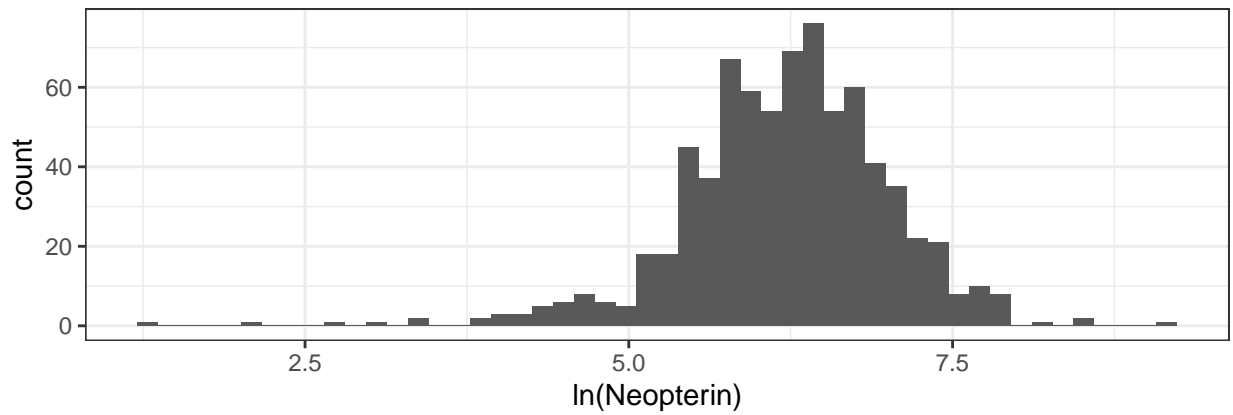
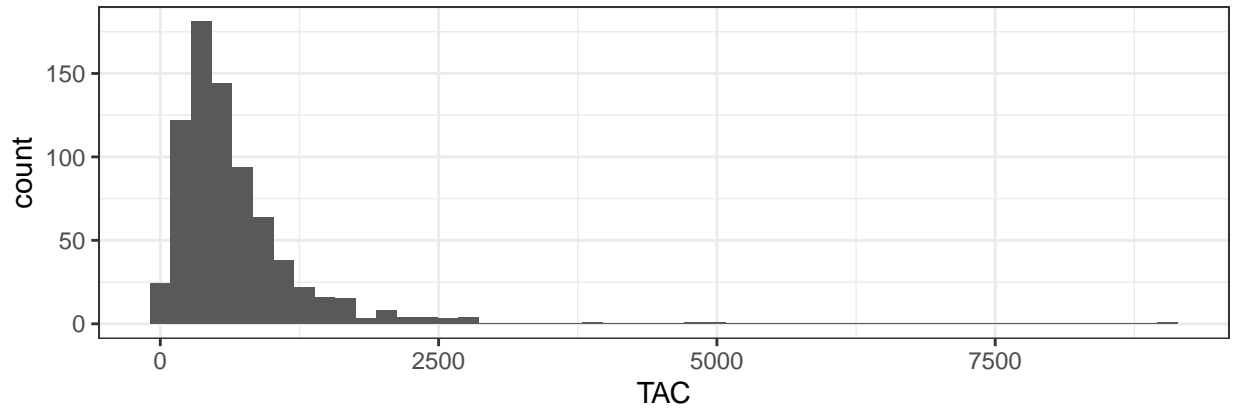


**TAC Variation** Variation in TAC seems to be the most interesting at first glance, as it is the most unique distribution. The units are in uM, not umol/L. But the transformation is a linear scale so the shape of the distribution will not change. It is possible that removing TAC values < 20uM might help, but the right tail is not too extreme. I am also reluctant to remove TAC values because they are so essential to interpreting oxidative stress. These high TAC values may be staving off (or failing to stave off) oxidative stress. If

8-OHdG is low during these high periods, it suggests the individual is dealing with something that is causing a lot of oxidative stress, but the body is otherwise successfully dealing with the problem as DNA damage does not increase. On the otherhand, if 8-OHdG is high in this upper range of TAC, it suggests the body is mounting a strong defense but it is not enough. I would want to check this. In addition, I want to see which individuals these high values are from, and if they have lower values as well or if some individuals just tend to produce higher values. Plot TAC over time by individual and see if any are typically high at all times. If such an individual exists, it is probably part of their own natural biology, and not indicative of chronic free radical generation.



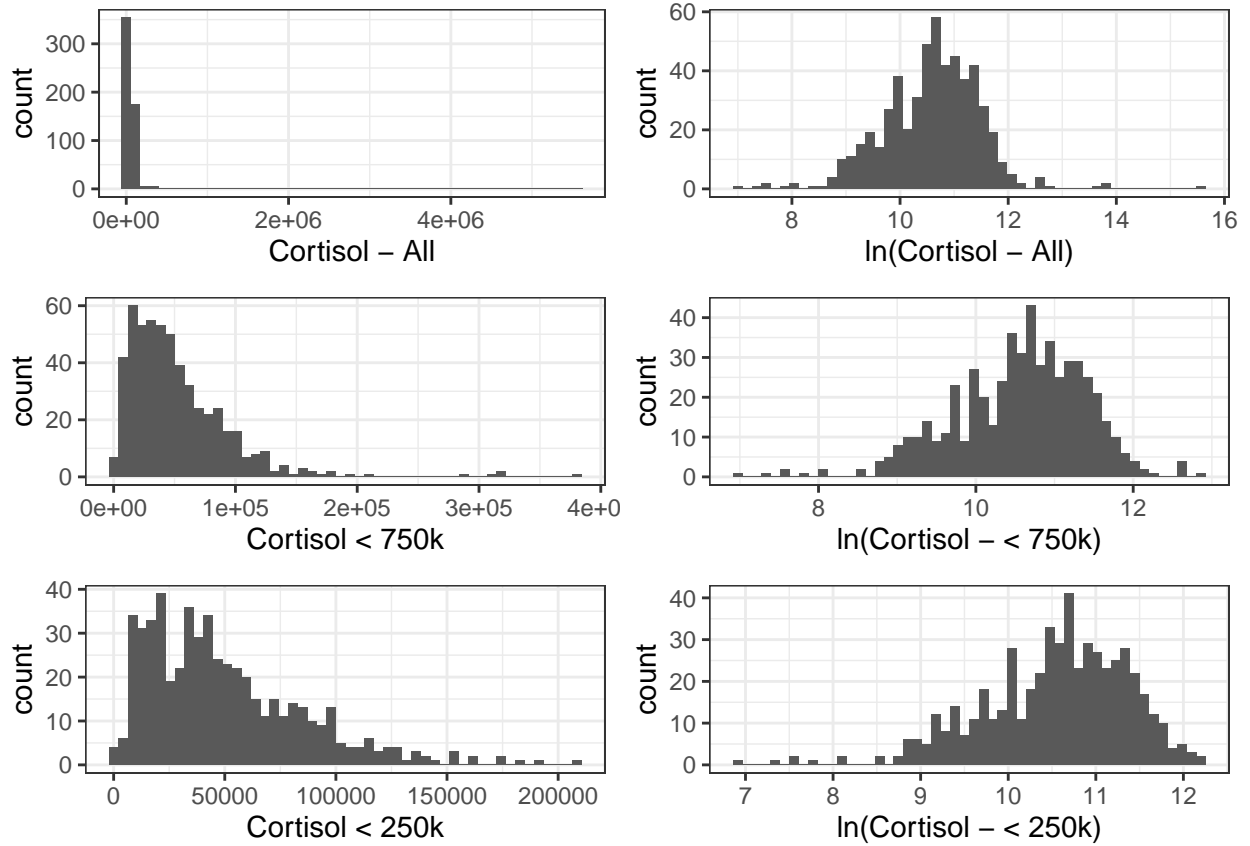
**Neopterin Variation** Neopterin, like cortisol below, has some pretty extreme outliers, though not to the same extent as cortisol. A natural log transformation does seem to bring these values together. However, I think I'd rather model the actual data using GAMMs as those values are much more interpretable than trying to understand the logged effects of biomarkers on each other.



**Cortisol Variation** You can see there are a small handful of extraordinarily extreme values of cortisol. They definitely need to be checked to determine if they are indeed correct. To temporarily view the distribution without these extreme values, I replicated the plots with X removed.

Table 4: Table: Skewness and Kurtosis of cortisol at different levels of outlier removal; only samples that meet inclusion criteria used

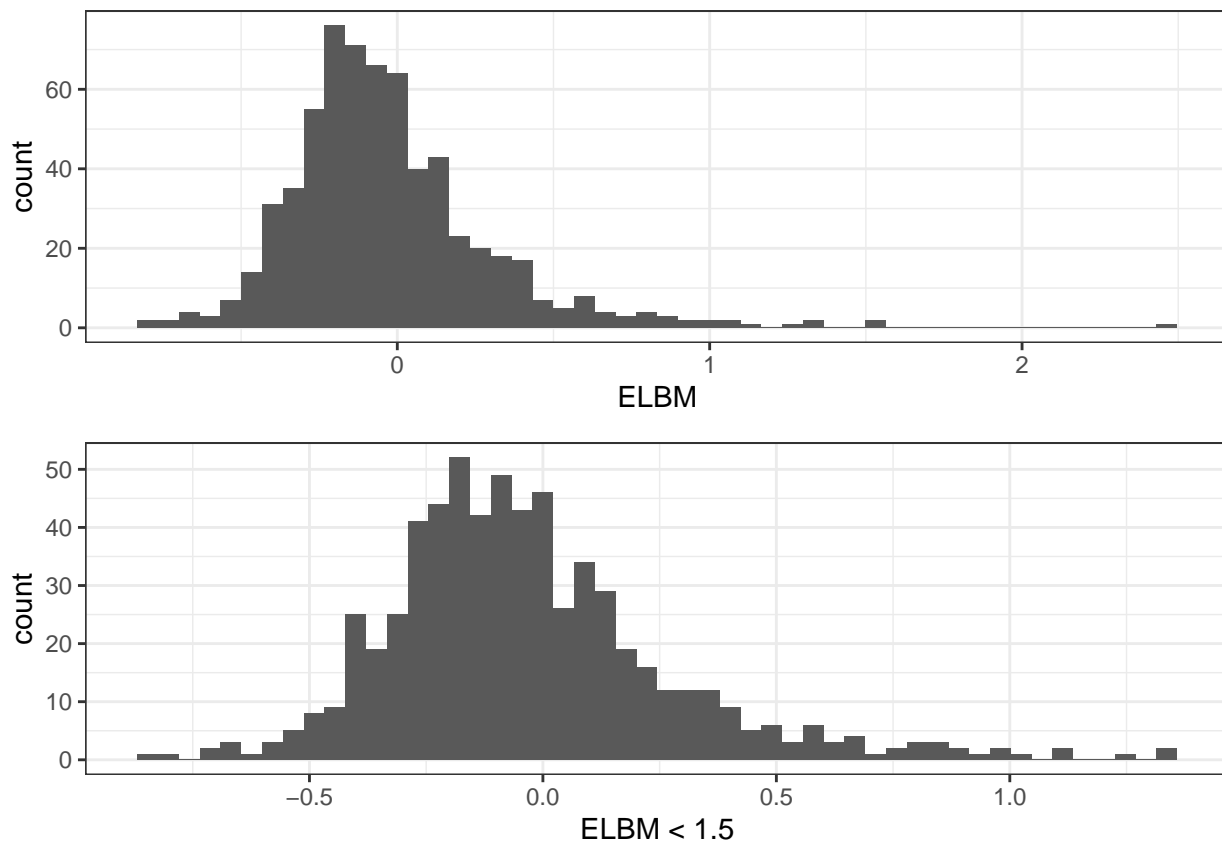
	Skewness	Kurtosis
All cortisol values	19.902720	432.612382
Cortisol < 750k	2.758784	16.210378
Cortisol < 250k	1.205298	4.656539



There is a pretty dramatic reduction in kurtosis when the most extreme outliers are removed. The reasoning for these outliers, be they error or some latent features, should be explored. In-depth exploration is probably beyond the scope of this paper; some decision must be made for which cortisol values to include and this decision must be justifiable.

**ELBM Distribution** I think I will keep in the outliers for now, and then see what to do about them later. *Note: as I was plotting covariation below, I routinely removed the ELBM outlier above 2*





**General Notes on Biomarker Distribution** There is some pretty heavy right tails in these data, regardless of how many outliers are removed. Special attention will need to be paid to these outliers given their sheer scale.

There is an outlier-robust GAM fitting package (rgam) that may be of use. It uses a backfitting algorithm with weights derived from robust quasi-likelihood equations. Original publication: Azadeh, A. and Salibian-Barrera, M. (2011). *An outlier-robust fit for Generalized Additive Models with applications to disease outbreak detection*.

## Outlier Analysis

Before proceeding beyond my analysis of variation of each of the biomarkers, I need to better understand what data points are outliers, why they are so different from the rest of the data, and then decide how to handle them. There are a range of techniques I could use to detect filters: z-score and flag points beyond the -3:3 z-score range, flag points outside the  $1.5 * \text{IQR}$  range, Expectation Maximization, Mahalanobis Distance, and DBSCAN. I will focus on the last 3 first as ML algorithms and compare their findings. I will likely stick with DBSCAN as it requires fewer assumptions, but I want to check first.

*Note: On returning to this point, I am not sure how much exploration is useful here or if avoiding it is more laziness. The ML approaches seem a bit overkill.*

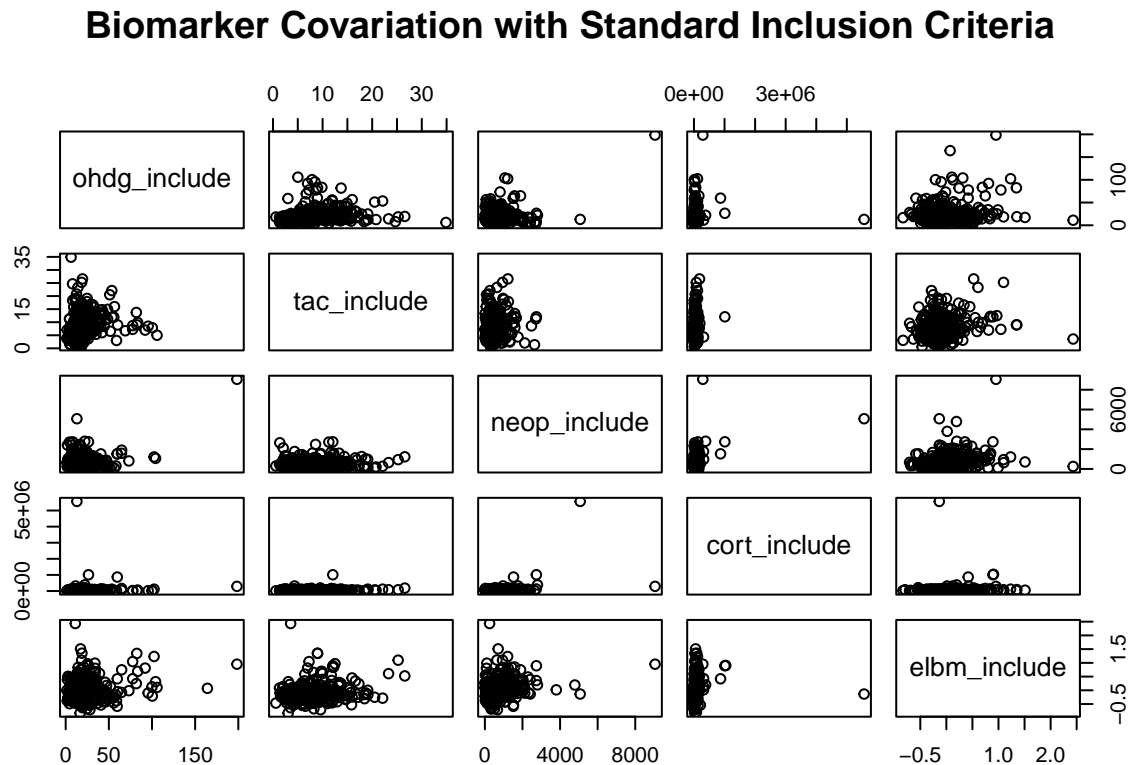
I want to flag each biomarker as an outlier under each method so that I can then determine if there is anything going on about them.

I created two markers for flagging each biomarker as an outlier. The first is based on  $-3:3$  z-score. The second arranges the values of a biomarker in ascending order and calculates the distance between successive points. My motivation was that the z-score (and IQR) method(s) seem very restrictive; several reasonable variables were cut out because the average of the biomarker was lower. The distance metric for 8-OHdG, for example, shows that the only data points that were very different from the values around it were the last two, which exhibited jumps of over 30 and 50, while all other distances were well below 5 or 10.

*Note: After looking at a lot of the distribution and box plots, I am going with the Z-score (aka  $\pm 3SD$ ) approach to identifying and removing outliers. Only the most extreme values are removed, and this approach is rather common among published papers. Work on the distance outliers can now be found in the supplement.*

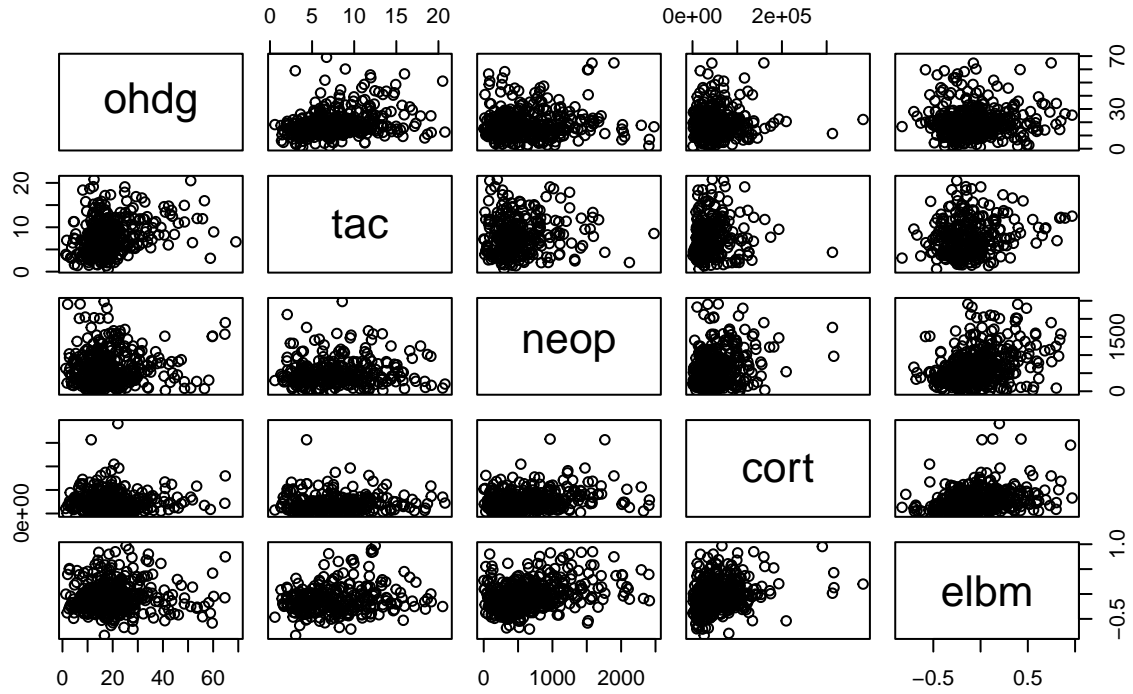
## Covariation

### Covariation between Biomarkers Alone



With outliers, it is difficult to determine any serious relationships.

## Biomarker Covariation with Z-Outliers Removed



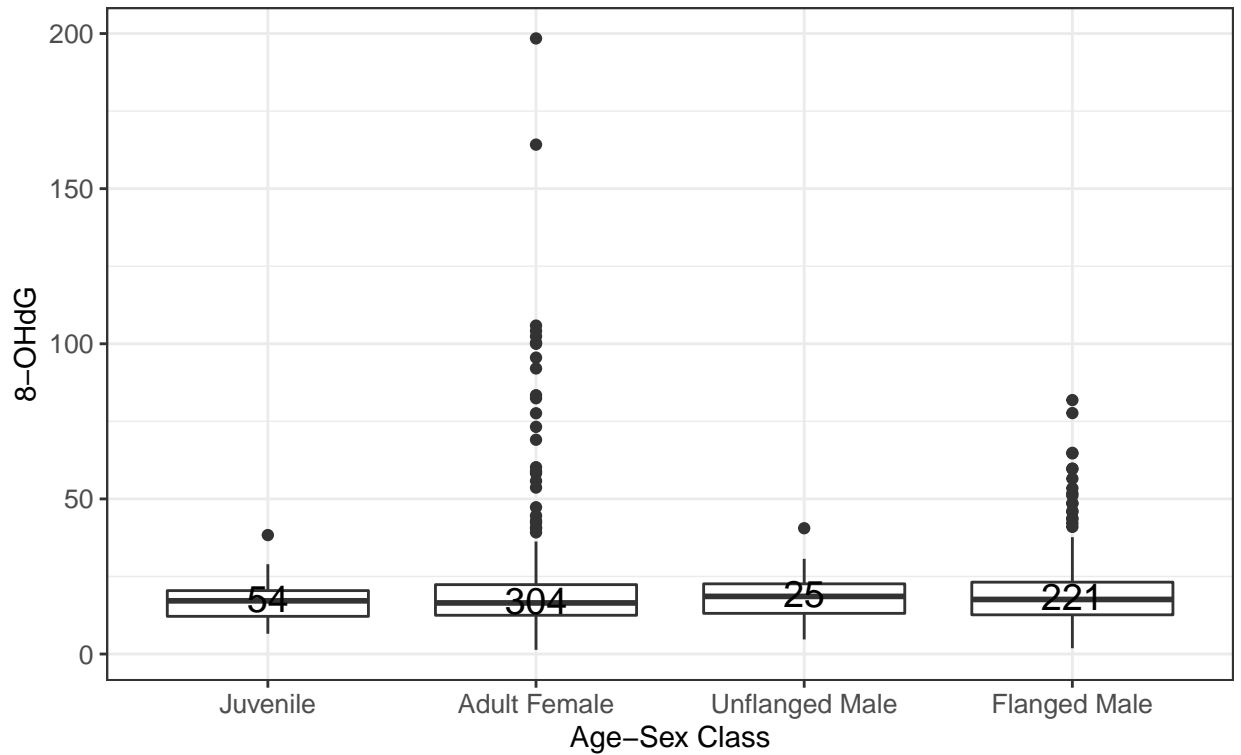
With outliers removed, several positive relationships do appear. 8-OHdG and TAC, TAC with maybe neopterin, 8-OHdG and neopterin, and ELBM with 8-OHdG, neopterin, and possibly cortisol.

## Covariation Between Biomarkers and Truncated Age-Sex Classes



## 8-OHdG with Standard Inclusion Criteria

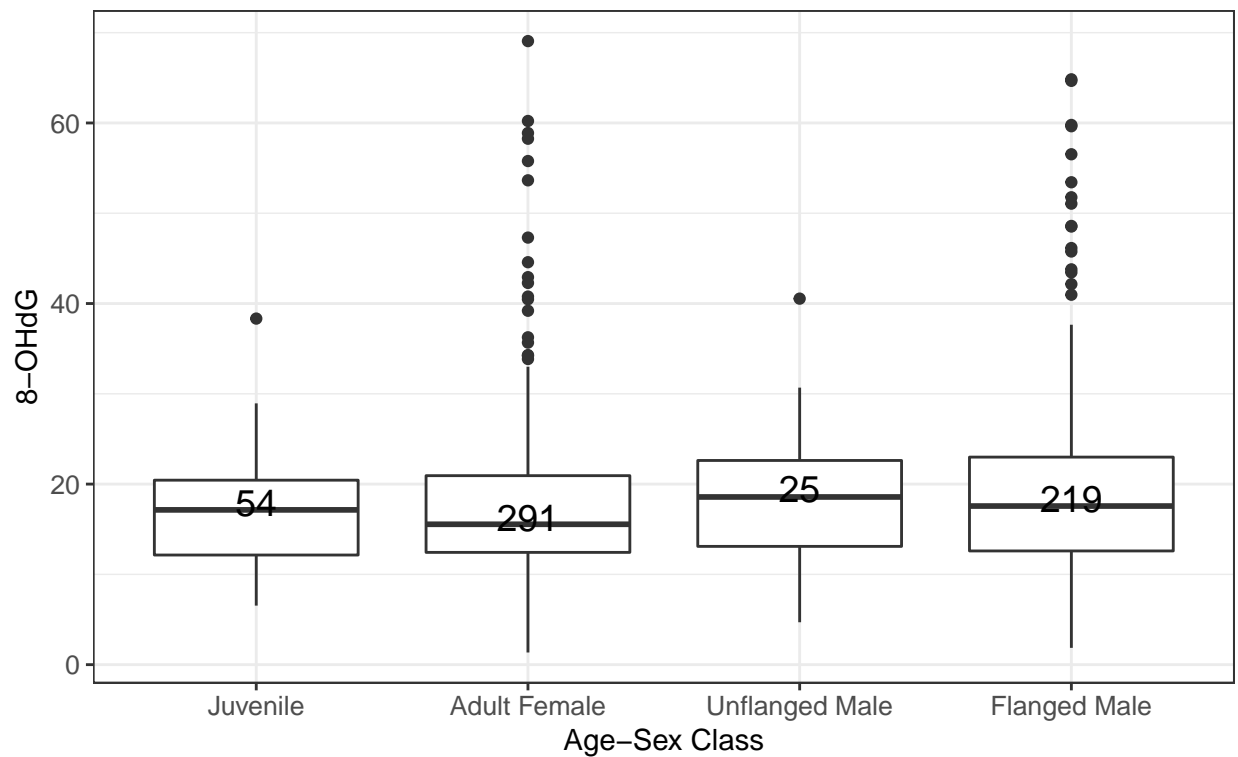
#s Indicate Urine Samples



8-OHdG

## 8-OHdG Z-Score Outliers Removed

#s Indicate Urine Samples

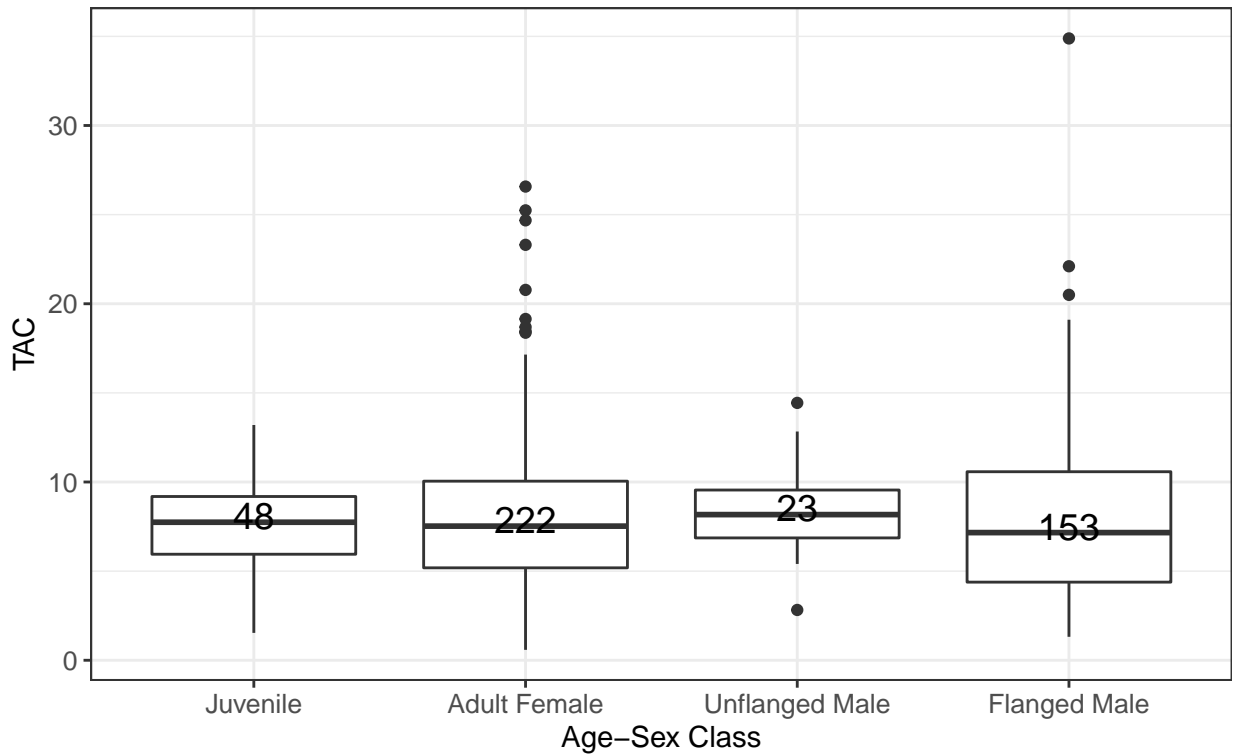


When looking at the samples with outliers removed, it almost looks like adult males may have higher 8-OHdG on average compared to females.



### TAC with Standard Inclusion Criteria

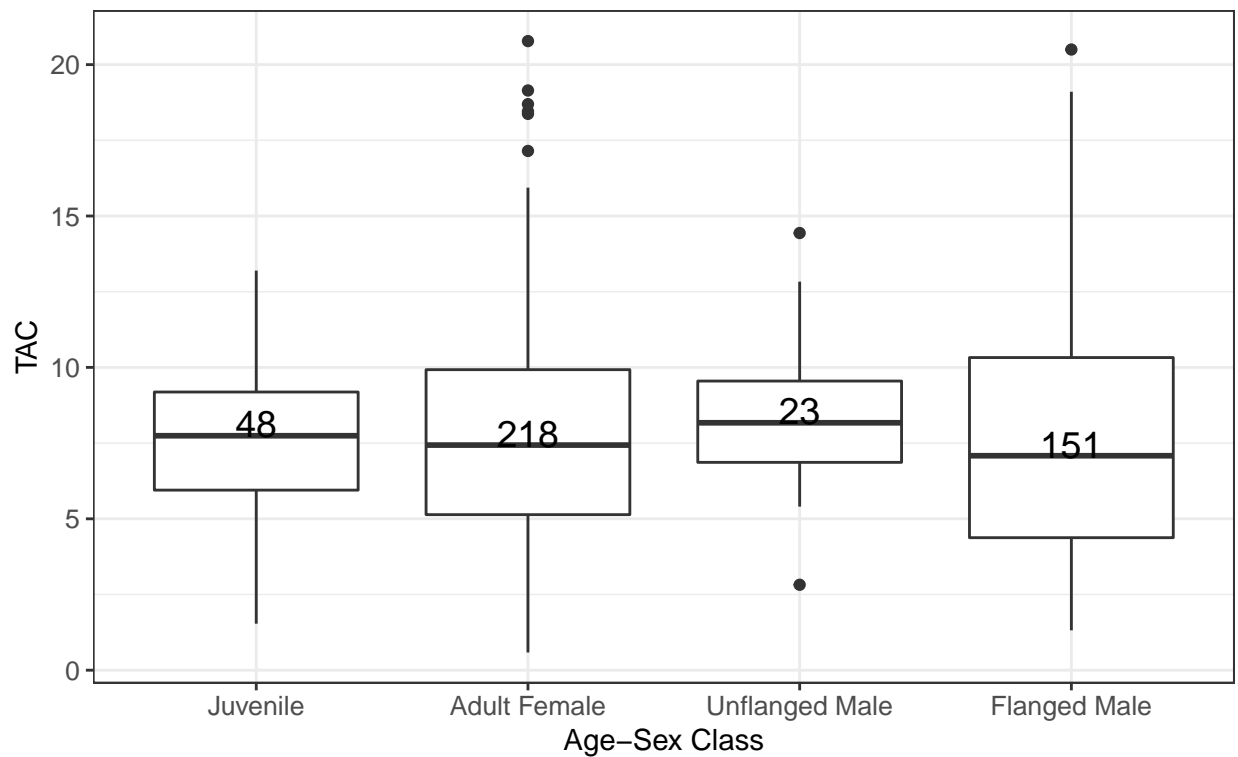
#s Indicate Urine Samples



TAC

### TAC Z-Score Outliers Removed

#s Indicate Urine Samples



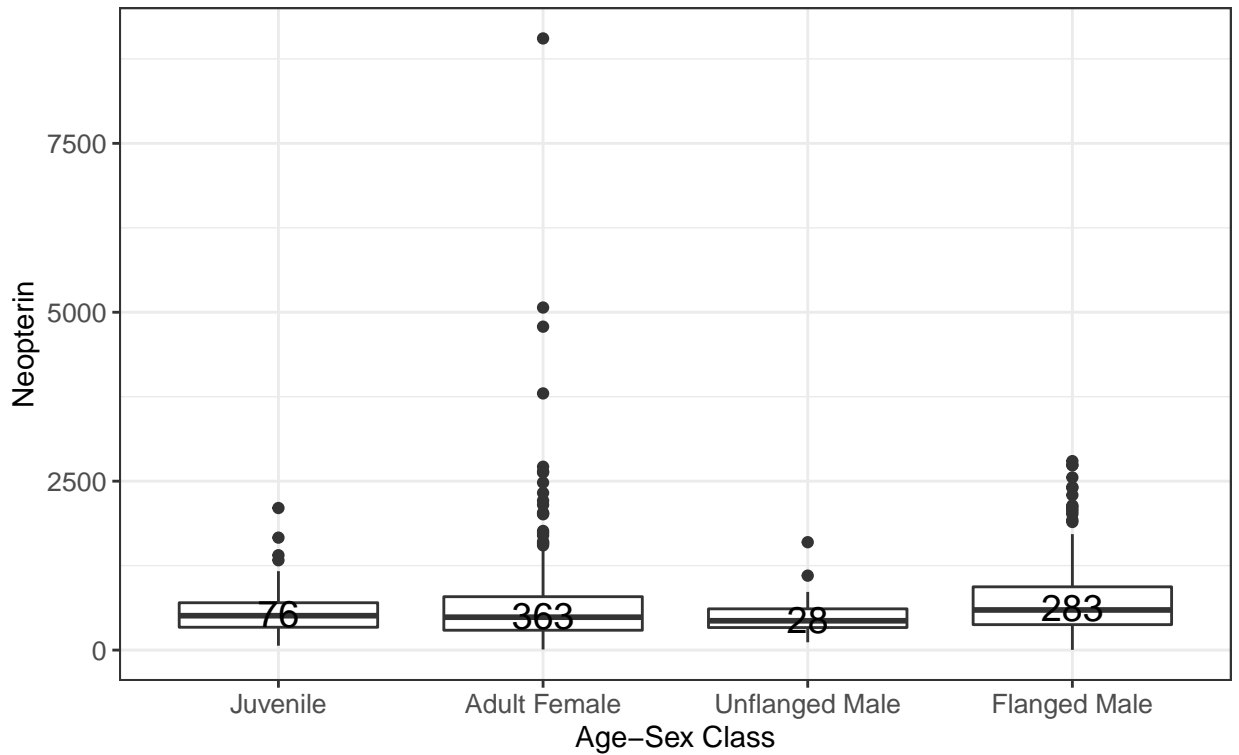


It looks like adult flanged males *might* have depressed antioxidants relative to unflanged males. However the difference in sample sizes (23 vs 151) warrants further exploration before any definitive conclusion is drawn. Some latent factor might drive this difference, especially given the range of unflanged male values falls entirely within that of the adult flanged males. Otherwise all groups seem to have a similar median and IQR.



### Neopterin with Standard Inclusion Criteria

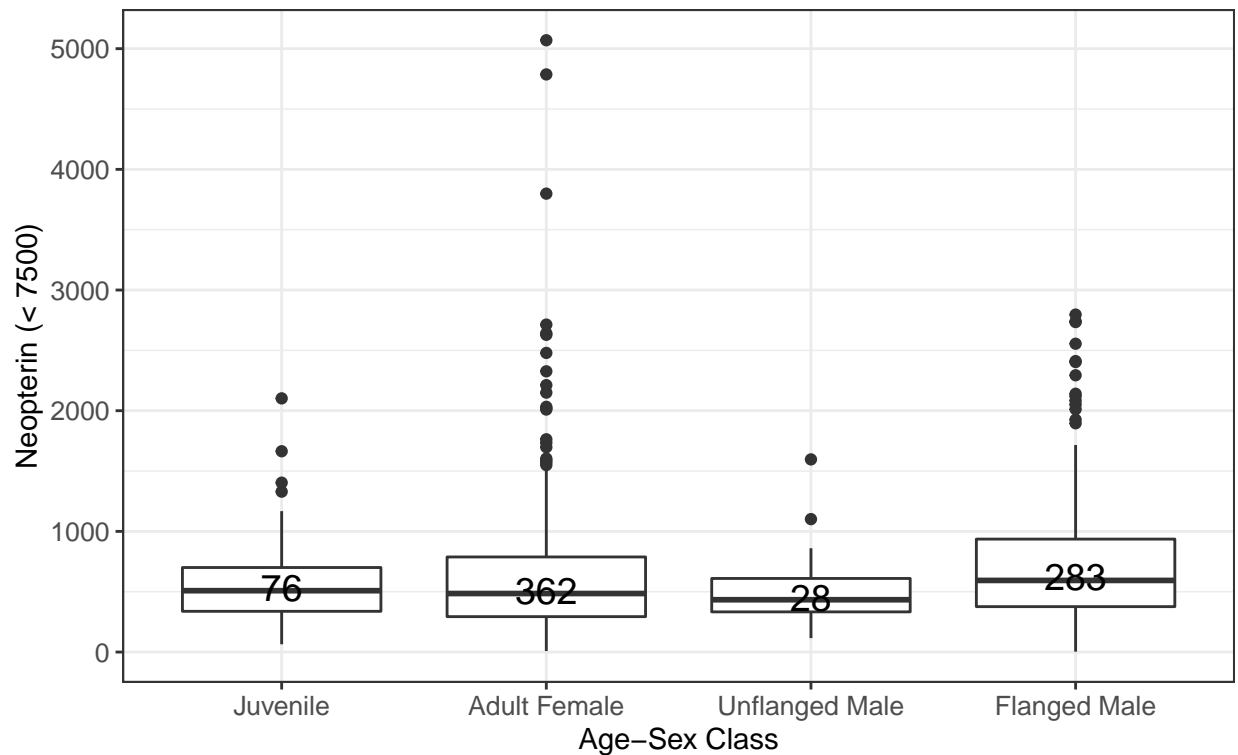
#s Indicate Urine Samples

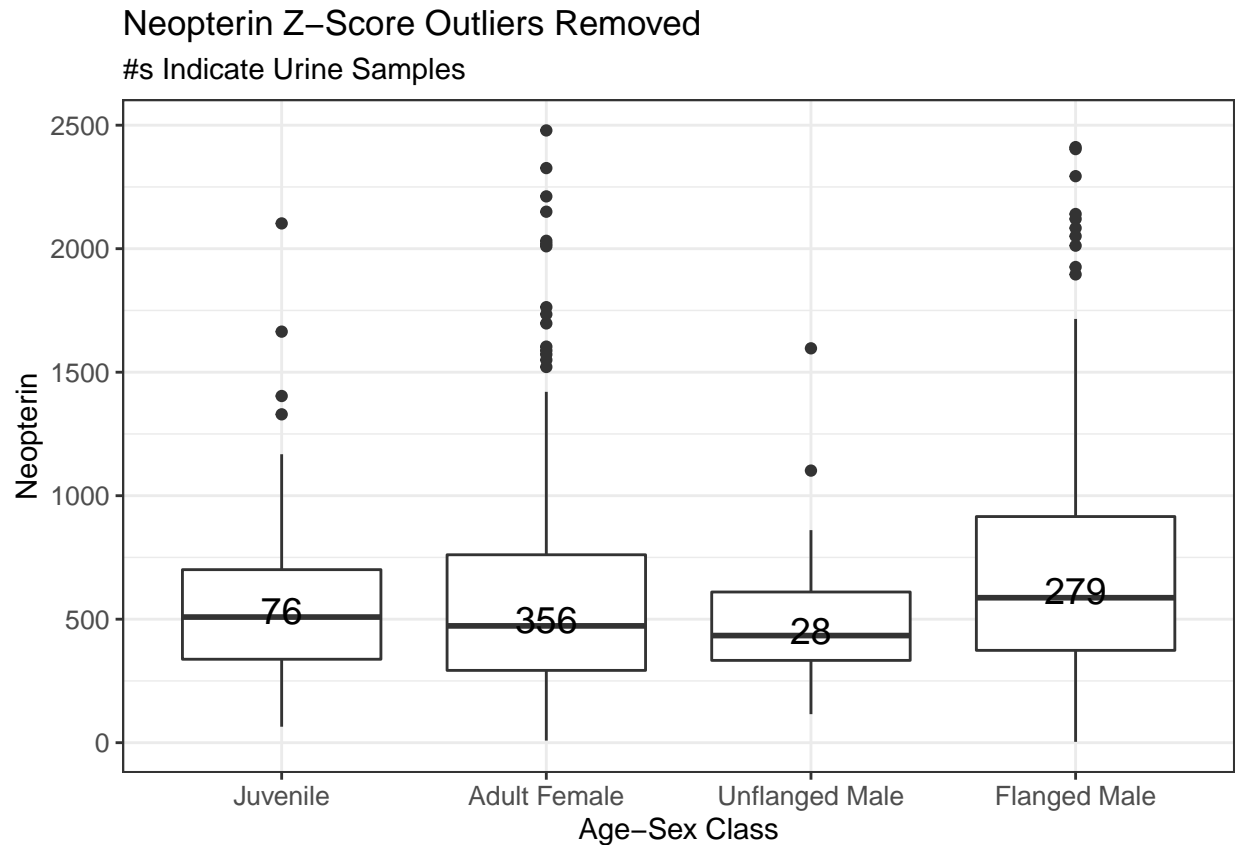


Neopterin

### Neopterin with Single High Outlier Removed

#s Indicate Urine Samples



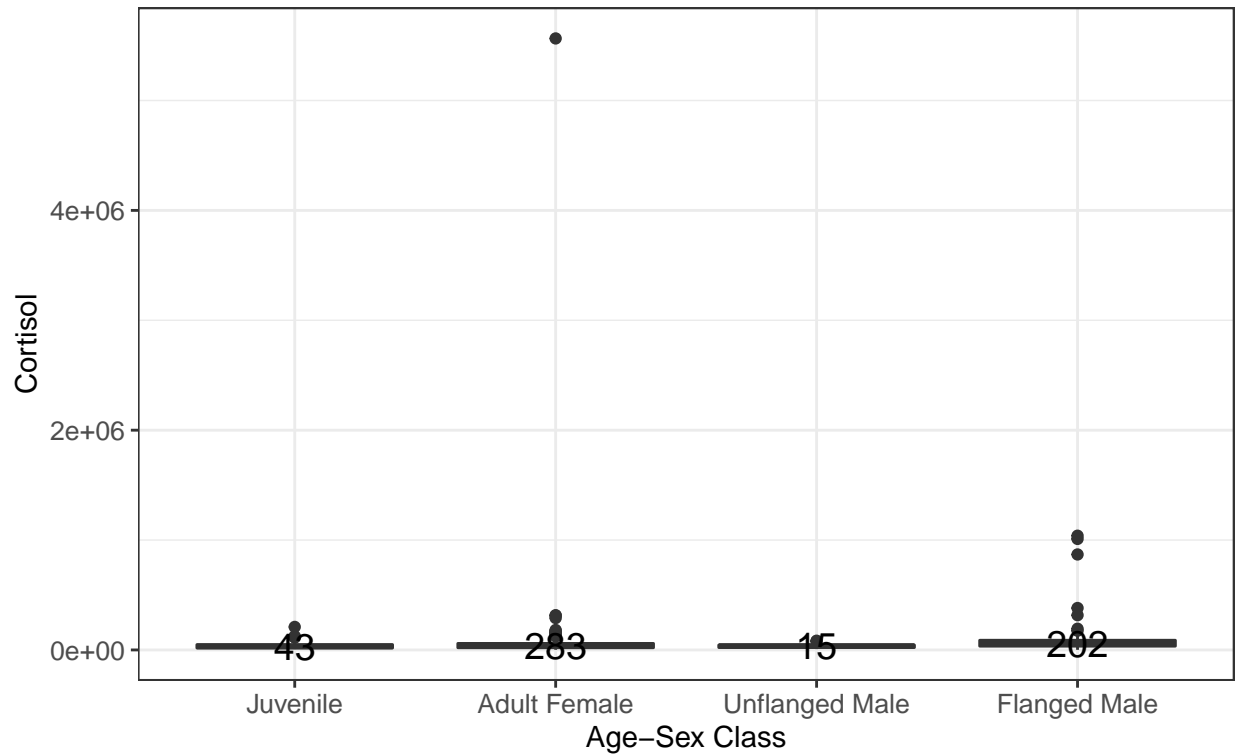


Looks like adult flanged males are dealing with greater inflammation on average when compared to adult females or adult unflanged males. This would probably fit with the idea that flanging is a costly process and the allocation of limited resources to growth and maintenance during adulthood pulls from the energy necessary to handle infections. Or perhaps something about the behavior of flanged males makes them more susceptible to infection. It is possible that behavioral differences might drive this pattern; possible explanations include flanged males 1) interact with other orangutans more frequently than unflanged males and adult females or 2) that they spend more time on the ground than unflanged males or adult females, then assessing the occurrence of these events with inflammation. Either of these might increase flanged male exposure to pathogens.



## Cortisol with Standard Inclusion Criteria

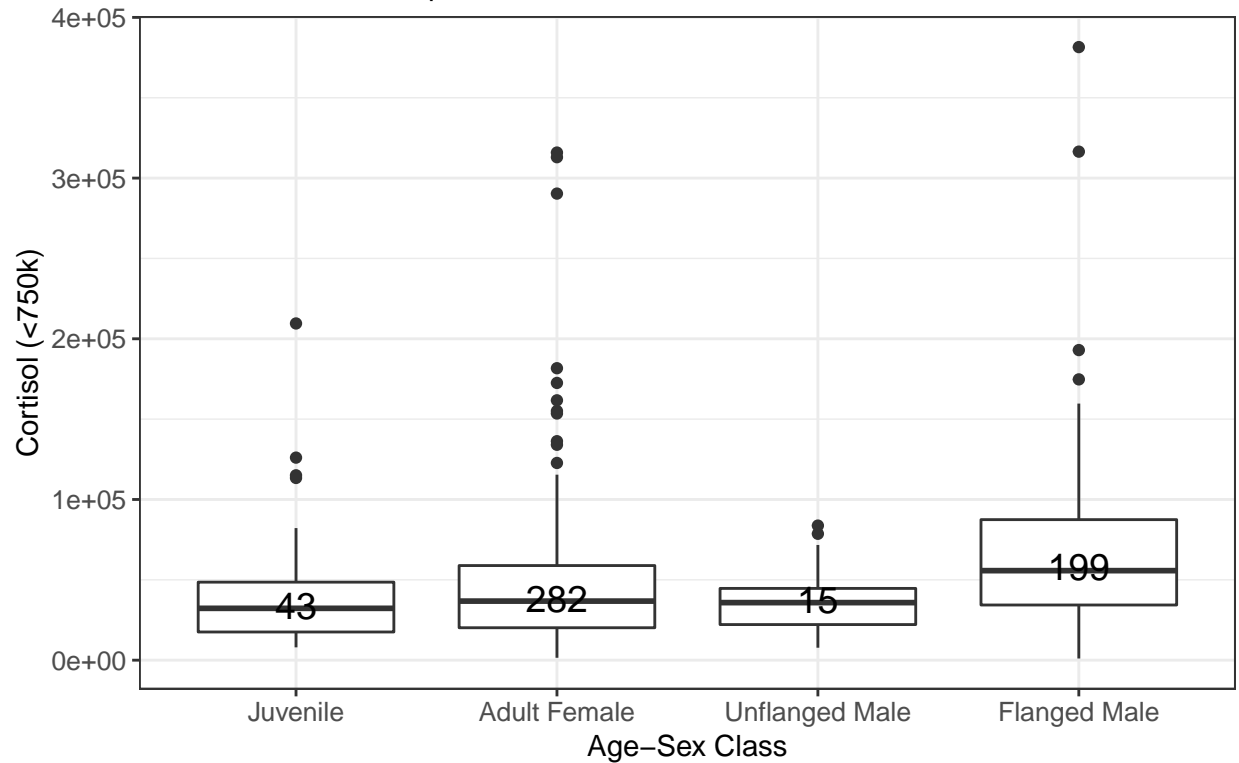
#s Indicate Urine Samples

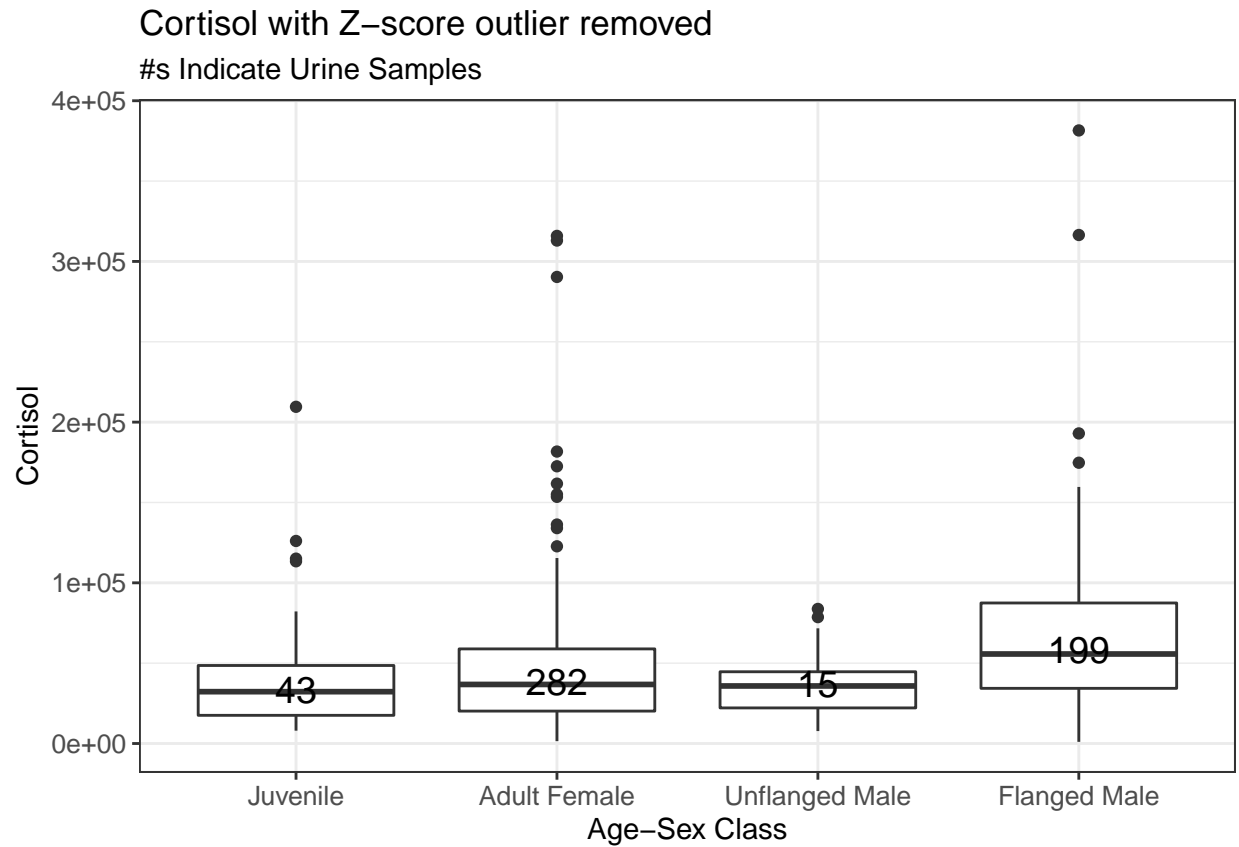


Cortisol

## Cortisol (one big outlier removed)

#s Indicate Urine Samples





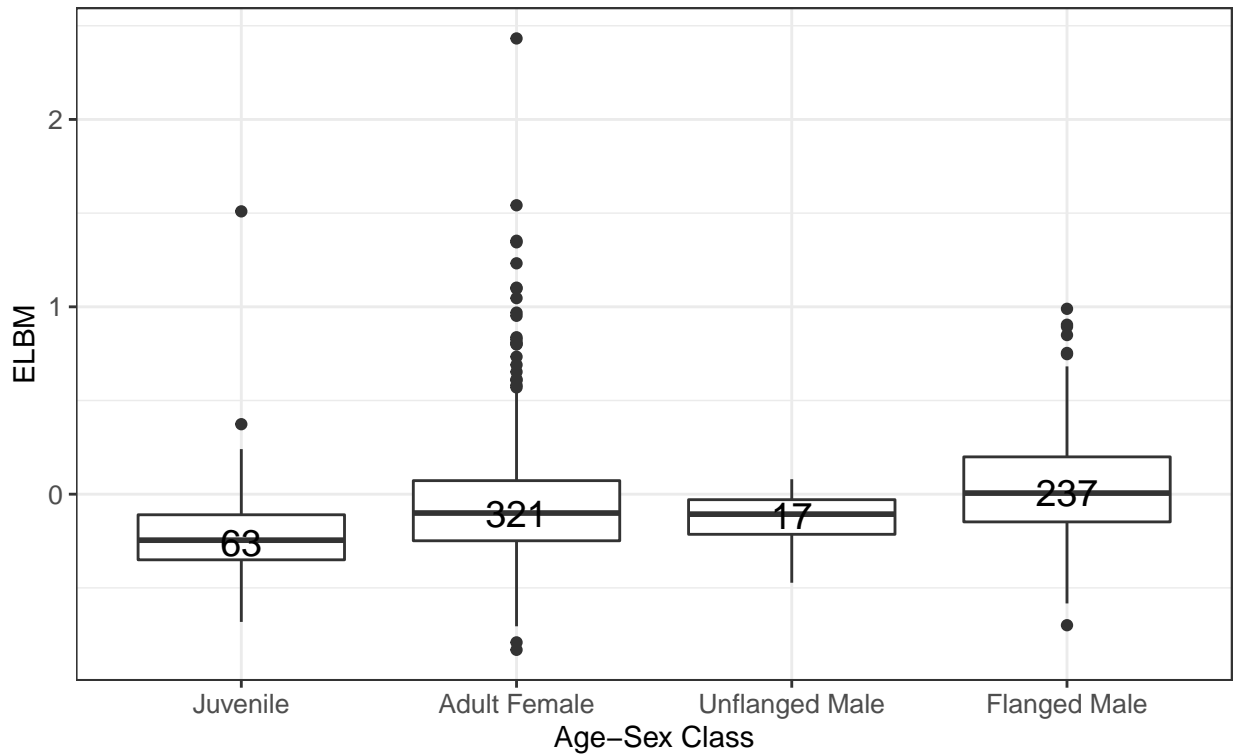
Looks like adult flanged males might be dealing with elevated cortisol relative to all other age-sex classes. Other age-classes have similar levels.





## ELBM Standard Inclusion Criteria

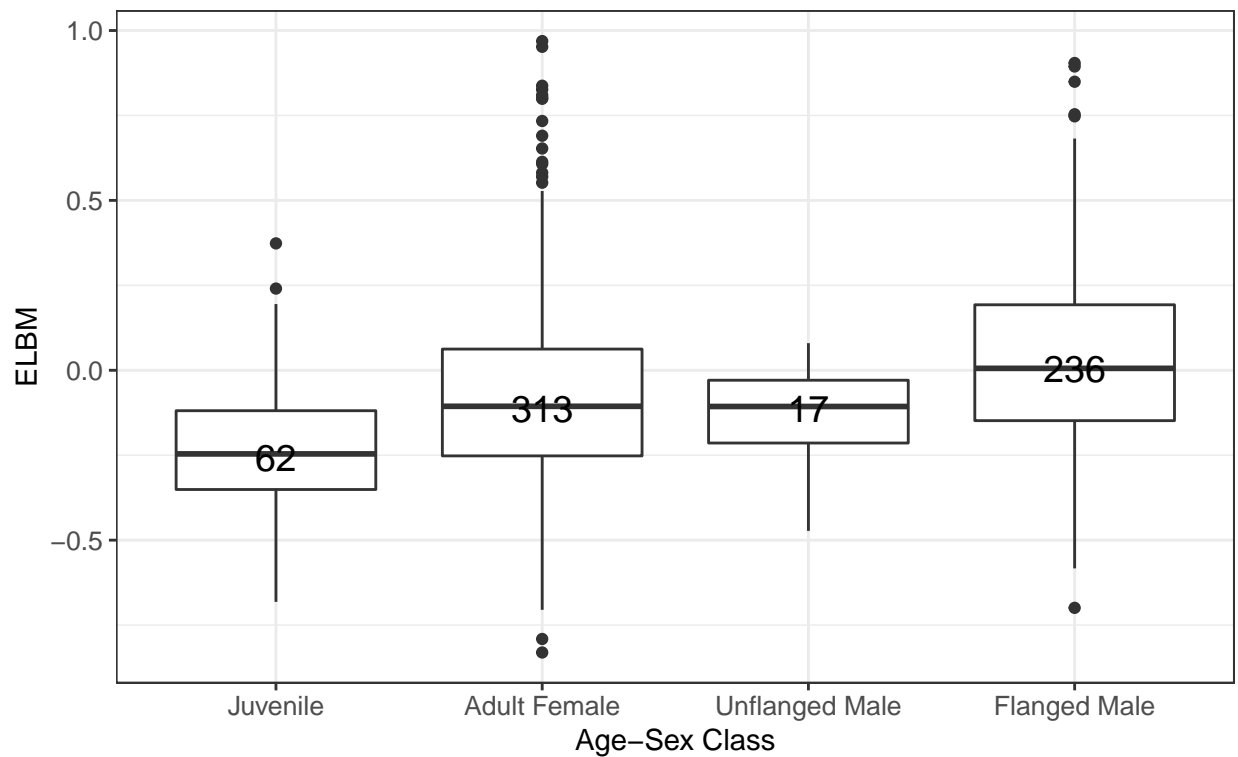
#s Indicate Urine Samples



ELBM

## ELBM Z-Score Outlier removed

#s Indicate Urine Samples



This is not new, basically partly replicated from O’Connell et al. 2021. I just wanted to visualize it within this report.

**Formally Test Difference in Biomarkers by Age-Sex class** Note that link functions for each response variable are determined in the supplement.

I am thinking I should either combine all dependents into a single category or a young male and young female category, and then probably merge the two adult female categories together.

I am following what was done in the O’Connell et al. 2021 paper, using GAMMs to test for significant differences between age-sex classes. She used gamm4, I will use mgcv::gamm. I will need to check about the distribution family that best works for each biomarker, potentially remove individuals with low sampling, and remove or group age-sex classes with very few data points, because data are quite unbalanced. currently have a random intercept by individual, not a random slope. There are few samples so this might not be possible. Note that Caitlin is exploring Neopterin, so any results of age-class ~ neopterin are just for funsies and will not be published.

## 8-OHdG

```
##
## Maximum number of PQL iterations: 20

##
## Family: gaussian
## Link function: log
##
## Formula:
## ohdg_include ~ Age_Cat_trunc
##
## Parametric coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)      2.99015    0.04315  69.301  <2e-16 ***
## Age_Cat_truncJuvenile -0.15543    0.09972  -1.559    0.120
## Age_Cat_truncAdult Female -0.09449    0.06296  -1.501    0.134
## Age_Cat_truncUnflanged Male -0.05276    0.12473  -0.423    0.672
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
## R-sq.(adj) = 0.00393
## Scale est. = 101.28    n = 589

## $‘emmeans of Age_Cat_trunc‘
## Age_Cat_trunc emmean SE df lower.CL upper.CL
## Flanged Male 19.7 0.867 37.2 18.0 21.5
## Juvenile 17.0 1.589 91.4 13.8 20.1
## Adult Female 18.1 0.881 18.1 16.2 19.9
## Unflanged Male 18.8 2.232 134.6 14.4 23.3
##
## Degrees-of-freedom method: kenward-roger
```

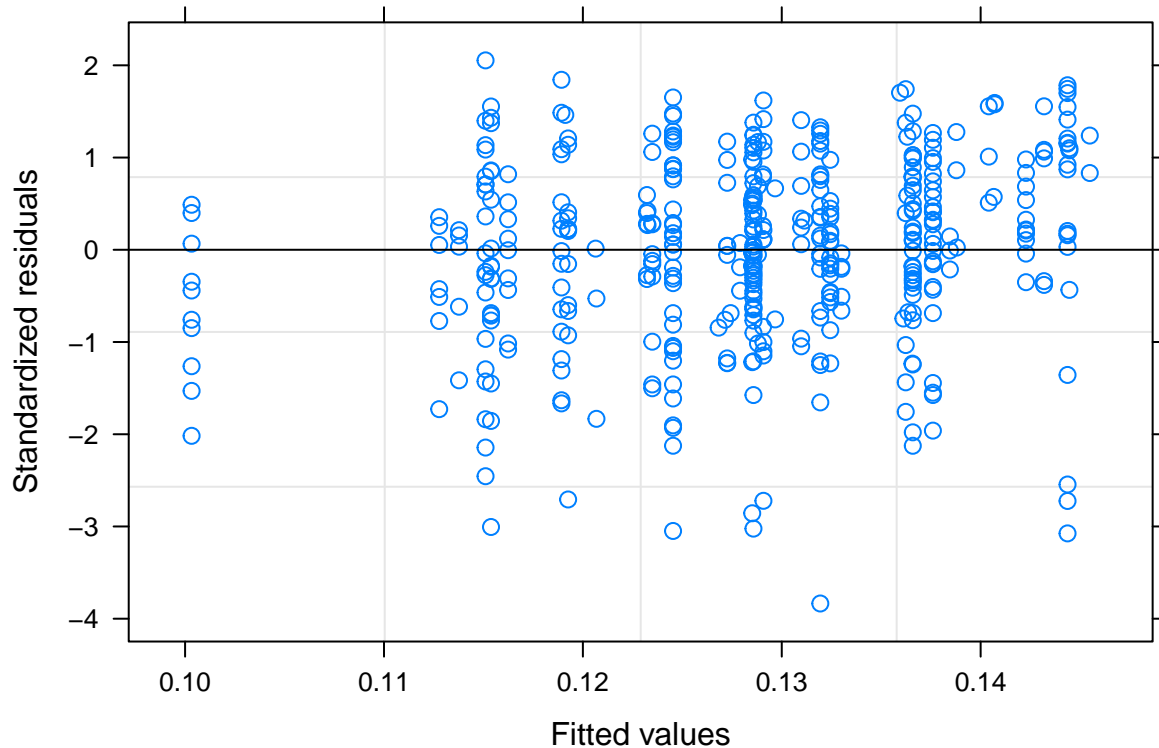
```
## Confidence level used: 0.95
##
## $'pairwise differences of Age_Cat_trunc'
## 1 estimate SE df t.ratio p.value
## Flanged Male - Juvenile 2.763 1.81 72.0 1.526 0.4274
## Flanged Male - Adult Female 1.659 1.24 25.1 1.342 0.5459
## Flanged Male - Unflanged Male 0.869 2.39 116.5 0.364 0.9834
## Juvenile - Adult Female -1.104 1.80 62.6 -0.613 0.9275
## Juvenile - Unflanged Male -1.893 2.74 117.5 -0.691 0.9003
## Adult Female - Unflanged Male -0.790 2.40 91.3 -0.329 0.9876
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 4 estimates
```

## TAC

```
##
## Maximum number of PQL iterations: 20

##
## Family: Gamma
## Link function: inverse
##
## Formula:
## tac_include ~ Age_Cat_trunc
##
## Parametric coefficients:
## Estimate Std. Error t value Pr(>|t|)
## (Intercept) 0.129991 0.006349 20.475 <2e-16 ***
## Age_Cat_truncJuvenile 0.008728 0.012588 0.693 0.488
## Age_Cat_truncAdult Female -0.005518 0.008903 -0.620 0.536
## Age_Cat_truncUnflanged Male -0.009398 0.014764 -0.637 0.525
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
## R-sq.(adj) = -0.00414
## Scale est. = 0.20618 n = 440

## [1] -1223.528
```



Well the standardized residuals vs fitted values are more homoscedastic and the AIC is lower in the model with outliers removed, but there is still at least one weird outlier. Only six samples ended up removed from this second model. Regardless, it would appear none of the age-sex categories are predictive of TAC. Not sure if this result would change if the categories were truncated.

## Neopterin

```
##
## Maximum number of PQL iterations: 20

##
## Family: gaussian
## Link function: log
##
## Formula:
## neop_include ~ Age_Cat_trunc
##
## Parametric coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    6.55171   0.03672 178.426 < 2e-16 ***
## Age_Cat_truncJuvenile -0.19085   0.09273  -2.058 0.039926 *
## Age_Cat_truncAdult Female -0.19476   0.05393  -3.611 0.000325 ***
## Age_Cat_truncUnflanged Male -0.30050   0.16079  -1.869 0.062037 .
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
## R-sq.(adj) =  0.0169
##   Scale est. = 1.8357e+05   n = 739

## $'emmeans of Age_Cat_trunc'
##   Age_Cat_trunc  emmean    SE    df lower.CL upper.CL
## Flanged Male      700 38.8  51.1     622      778
## Juvenile          571 65.7 100.9     441      702
## Adult Female      590 43.6  31.4     501      679
## Unflanged Male    520 95.3 153.4     332      708
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $'pairwise differences of Age_Cat_trunc'
##   1 estimate    SE    df t.ratio p.value
## Flanged Male - Juvenile      128.5  76.3  82.8   1.685  0.3382
## Flanged Male - Adult Female   110.3  58.4  38.4   1.889  0.2497
## Flanged Male - Unflanged Male 179.9 101.8 143.1   1.767  0.2936
## Juvenile - Adult Female     -18.3  76.9  79.2  -0.237  0.9953
## Juvenile - Unflanged Male    51.4 115.7 133.1   0.444  0.9706
## Adult Female - Unflanged Male  69.7 104.8 106.7   0.665  0.9101
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 4 estimates
```

Adult flanged males have the higher neopterin than other groups. Not significant when looking at the linear model. Still not sure how the emmeans vs lmerTest p-values differ or how to really interpret them. Based on the emmeans and associated confidence intervals, FM are on average higher but the confidence intervals overlap a lot. Suggests age might not be a driver.

## Cortisol

```
##
## Maximum number of PQL iterations: 20
##
## Family: Gamma
## Link function: inverse
##
## Formula:
## cort_include ~ Age_Cat
##
## Parametric coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  2.759e-05  2.148e-05   1.284   0.200
## Age_CatDINC -1.026e-05  2.278e-05  -0.450   0.653
## Age_CatSII   1.960e-06  2.297e-05   0.085   0.932
## Age_CatII    -6.392e-06  2.179e-05  -0.293   0.769
```

```
## Age_CatUFM -1.406e-06 2.220e-05 -0.063 0.950
## Age_CatAFM -1.262e-05 2.153e-05 -0.586 0.558
## Age_CatAFnD -9.922e-06 2.190e-05 -0.453 0.651
## Age_CatAFwD -6.117e-06 2.155e-05 -0.284 0.777
##
##
## R-sq.(adj) = 0.0349
## Scale est. = 0.57043 n = 539
```

Some large standardized residuals. At first glance it would appear that flanged males are more stressed, but not by a lot.

## Additional Supplementary Information

### Determine What Link Function is Best for GAMMs for Each Biomarker

For each of the 4 distributions (norm, lnorm, gamma, exp), four plots are produced along with parameter estimates, log likelihood, AIC, and BIC. The four plots are:

- a histogram of data (empirical) against fitted density function
- Q-Q Plot: theoretical quantiles against empirical quantiles
- empirical cumulative distribution against fitted distribution function
- P-P plot :theoretical probabilities against empirical probabilities

See the following text from the vignette for the `fitdistrplus` package Found at: <https://cran.r-project.org/web/packages/fitdistrplus/vignettes/FAQ.html>

*Goodness-of-fit tests often appear as objective tools to decide whether a fitted distribution well describes a data set. But they are not ! It would not be reasonable at all to reject a distribution just because a goodness-of-fit test rejects it (see FAQ 2.2.1). And it would not be reasonable at all any more to validate a distribution because goodness-of-fit tests do not reject it (see FAQ 2.2.2).*

*A fitted distribution should be evaluated using graphical methods (goodness-of-fit graphs automatically provided in our package by plotting the result of the fit (output of `fitdist()` or `fitdistcens()`) and the complementary graphs that help to compare different fits - see `?graphcomp`). We really think it is the most appropriate way to evaluate the adequacy of a fit and we are not the only ones to recommend it. You can find the same type of recommendations in reference books:*

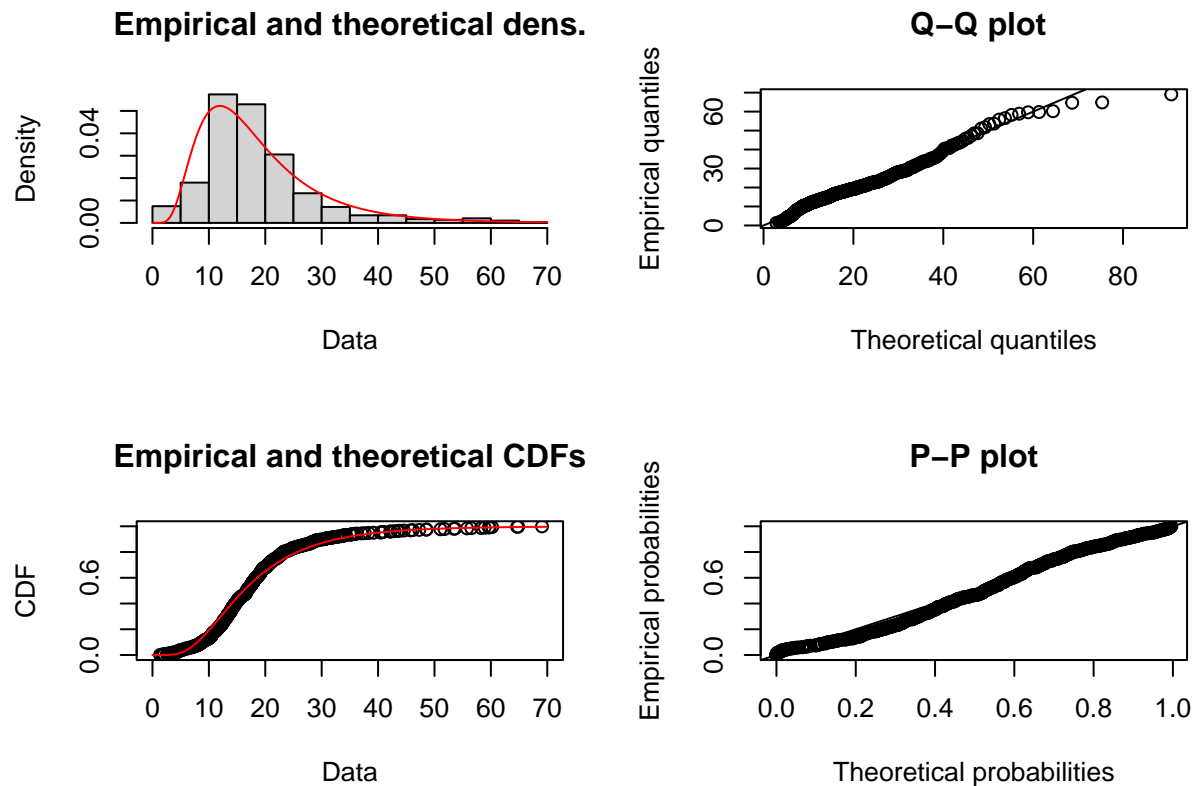
- Probabilistic techniques in exposure assessment - a handbook dealing with variability and uncertainty in models and inputs by A.C. Cullen and H.C. Frey.
- Application of uncertainty analysis to ecological risks of pesticides by W.J. Warren-Hicks and A. Hart.
- Statistical inference by G. Casella and R.L. Berger
- Loss models: from data to decision by S.A. Klugman and H.H. Panjer and G.E. Willmot

## 8-OHdG

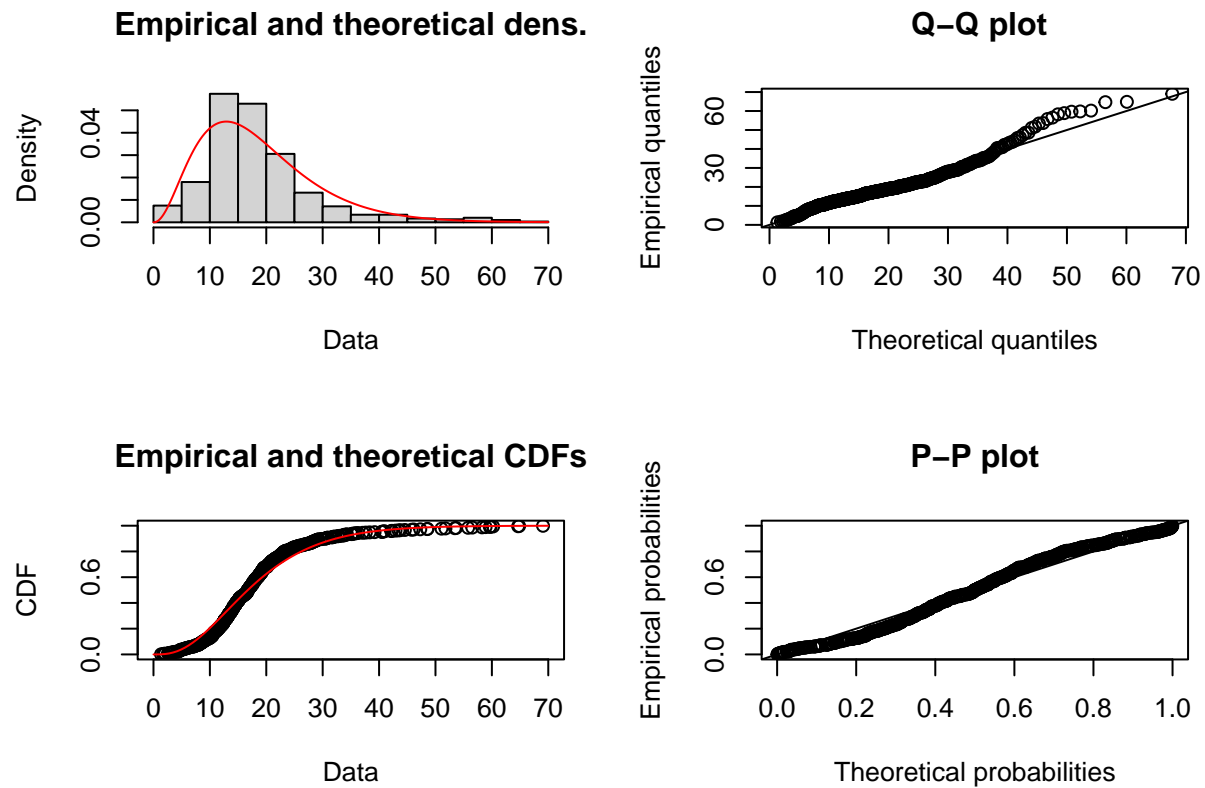
**Standard Inclusion Criteria** Note: log-normal fits best but there are two substantial outliers. Plots excluded.

**Z-Score Outliers Removed** Note: Based on graphs, log-normal fits best, with gamma a close second. Looking at AIC and BIC, it would seem the Gamma distribution is best. I'm not yet sure which I want to go with. log-normal's P-P plot and Empirical/Theoretical CDFs are a tighter fit to the line their respective curves. Given what the authors say about using graphics, I am leaning choosing log-normal.

```
## Fitting of the distribution 'lnorm' by maximum likelihood
## Parameters :
##      estimate Std. Error
## meanlog 2.7823054 0.02265424
## sdlog    0.5498031 0.01601873
## Loglikelihood: -2122.196   AIC:  4248.391   BIC:  4257.148
## Correlation matrix:
##      meanlog sdlog
## meanlog      1      0
## sdlog        0      1
```



```
## Fitting of the distribution 'gamma' by matching moments
## Parameters :
##      estimate
## shape 3.2799364
## rate  0.1765455
## Loglikelihood: -2115.013   AIC:  4234.025   BIC:  4242.782
```



## TAC

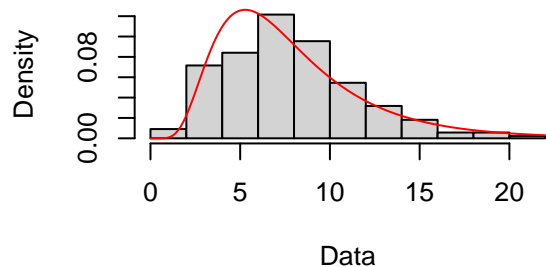
**Standard Inclusion Criteria** Note: it looks like gamma fits best, with log-normal a close second. Plots excluded.

**Z-Score Outliers Removed** Note: Gamma is an excellent fit

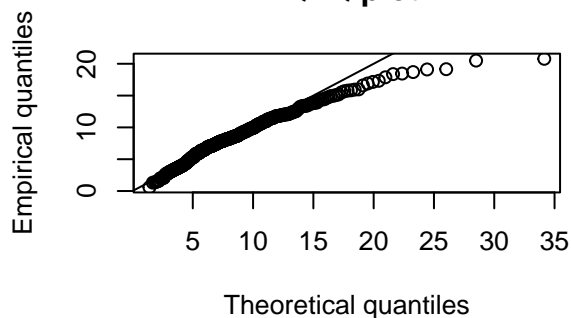
```
## Fitting of the distribution 'lnorm' by maximum likelihood
## Parameters :
##      estimate Std. Error
## meanlog 1.9362087 0.02490563
## sdlog    0.5224249 0.01761065
## Loglikelihood: -1190.584   AIC:  2385.168   BIC:  2393.342
## Correlation matrix:
##      meanlog sdlog
## meanlog      1      0
## sdlog         0      1
```



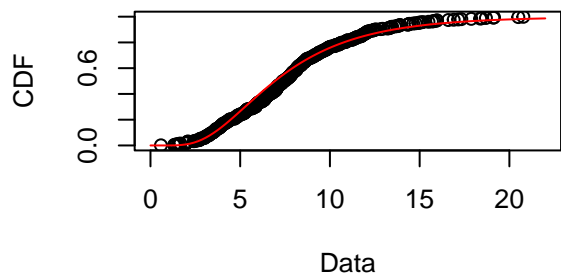
**Empirical and theoretical dens.**



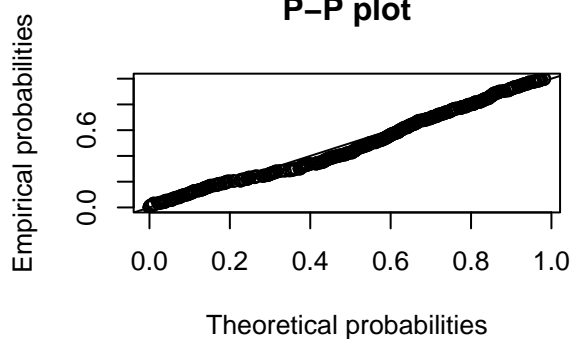
**Q-Q plot**



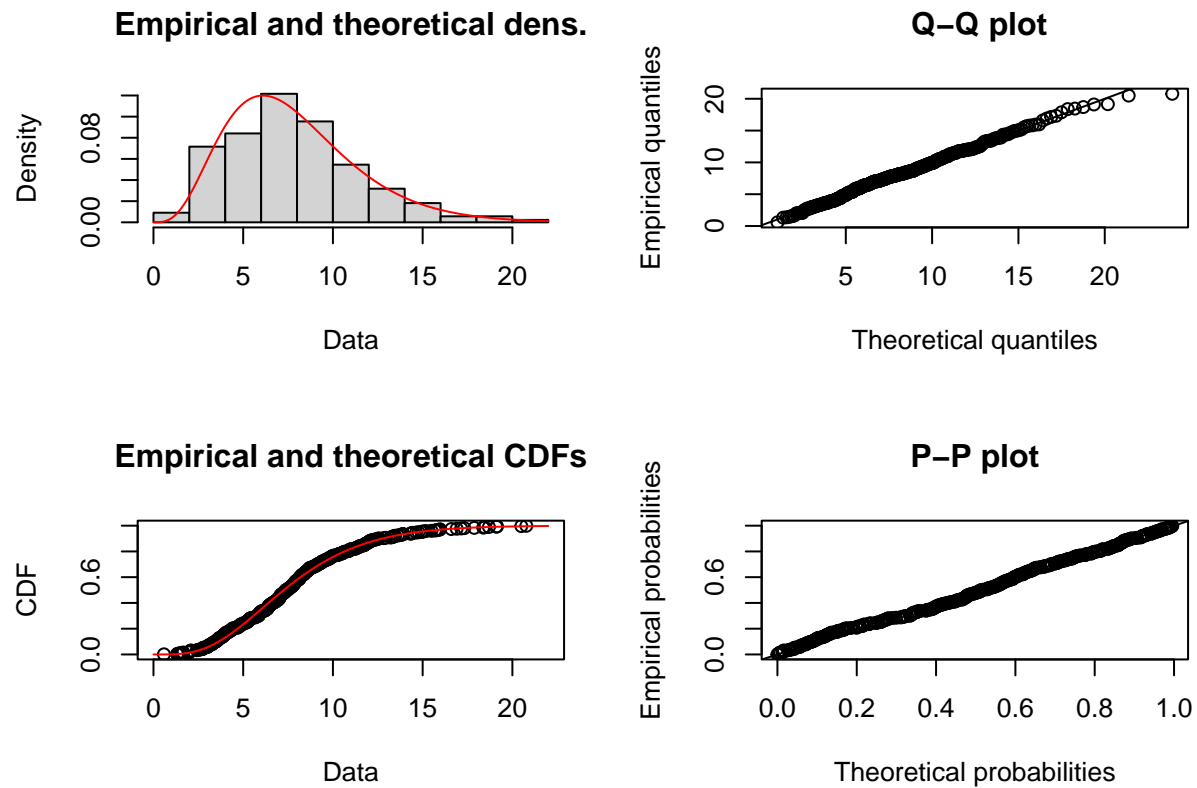
**Empirical and theoretical CDFs**



**P-P plot**



```
## Fitting of the distribution ' gamma ' by matching moments
## Parameters :
##      estimate
## shape 4.5221421
## rate  0.5775776
## Loglikelihood: -1174.514   AIC:  2353.028   BIC:  2361.202
```



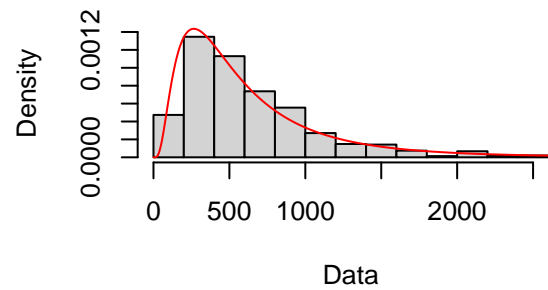
## Neopterin

**Standard Inclusion Criteria** Note: log-normal fits best, although there is at least one substantial outlier, with the potential for a few other smaller outliers. Plots excluded.

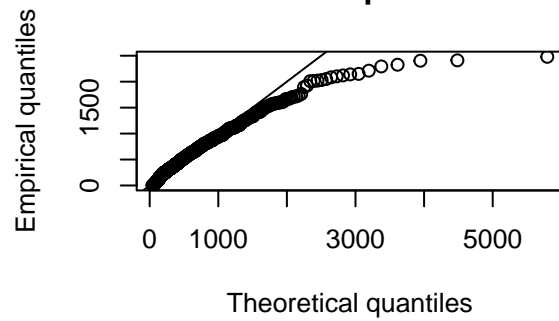
**Z-Score Outliers Removed** Note: Gamma is best; log-normal is **not** great

```
## Fitting of the distribution 'lnorm' by maximum likelihood
## Parameters :
##      estimate Std. Error
## meanlog 6.1828257 0.02849262
## sdlog    0.7745591 0.02014717
## Loglikelihood: -5428.918   AIC: 10861.84   BIC: 10871.05
## Correlation matrix:
##      meanlog sdlog
## meanlog      1      0
## sdlog         0      1
```

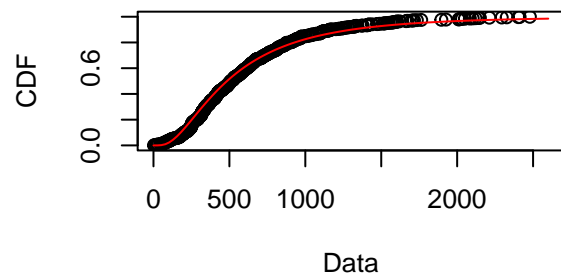
**Empirical and theoretical dens.**



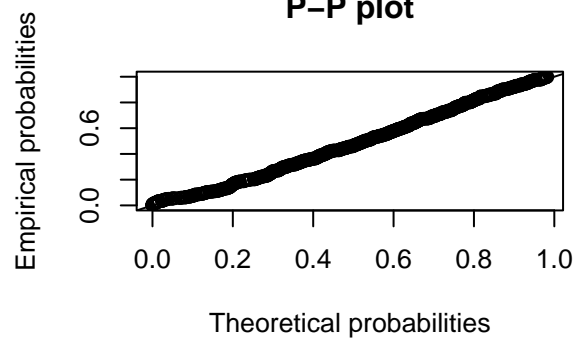
**Q-Q plot**



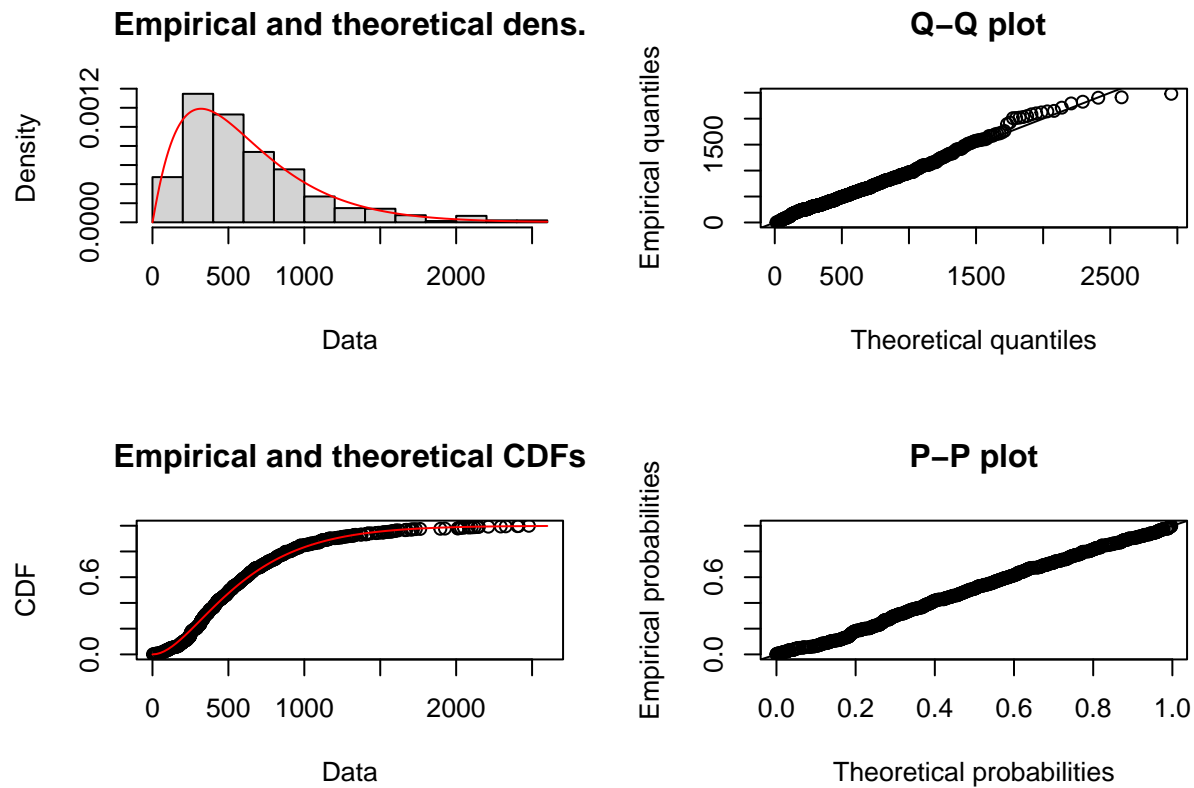
**Empirical and theoretical CDFs**



**P-P plot**



```
## Fitting of the distribution ' gamma ' by matching moments
## Parameters :
##      estimate
## shape 2.05904565
## rate 0.00331395
## Loglikelihood: -5389.957   AIC: 10783.91   BIC: 10793.12
```

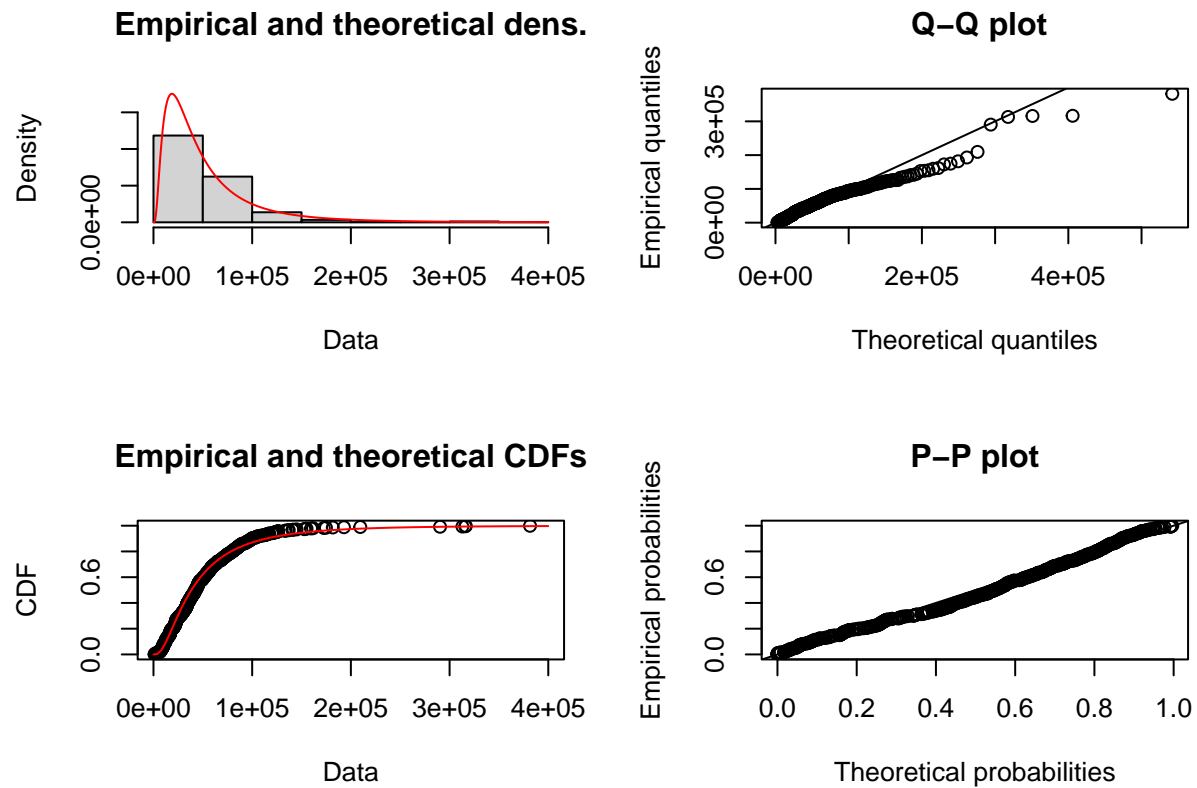


## Cortisol

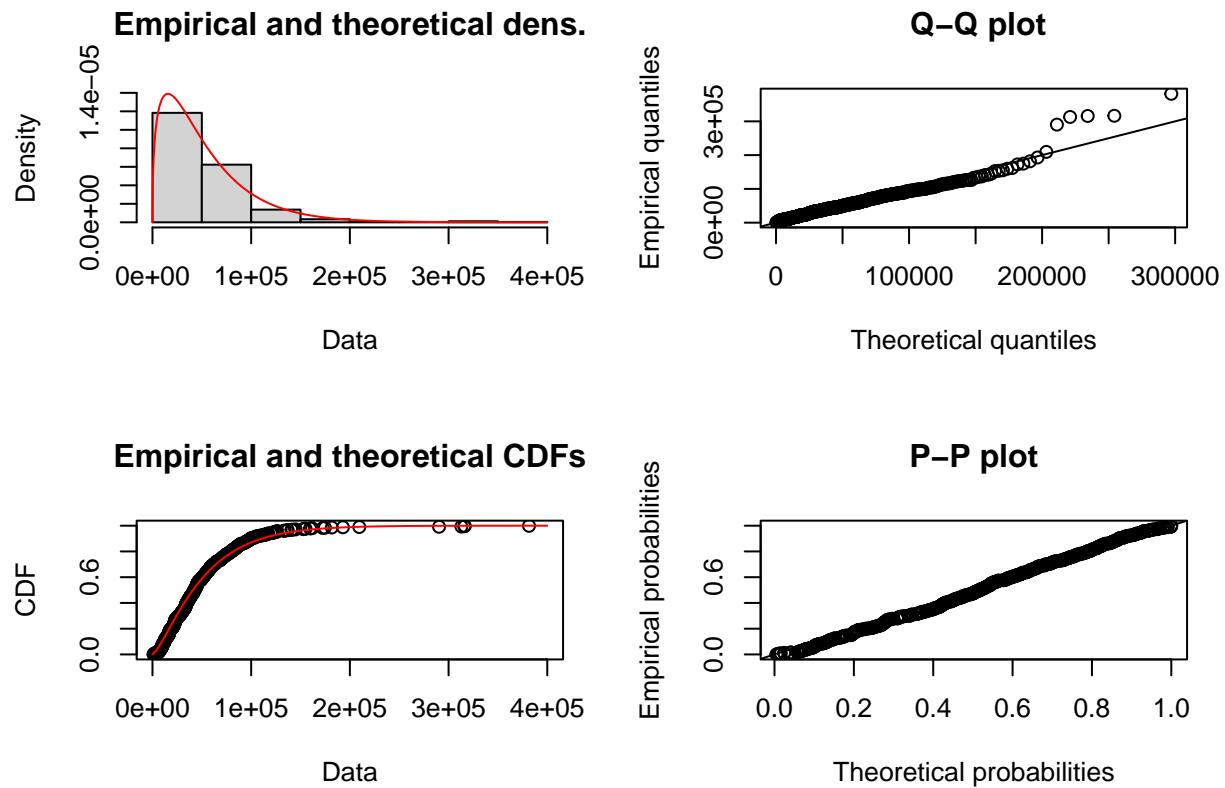
**Standard Inclusion Criteria** Note: log-normal fits best but there are a few outliers, one of which is quite extreme. Plots excluded.

**Z-Score Outliers Removed** Note: Gamma fits best, although I would like to consider removing between 1 and 5 outliers to improve the Q-Q plot fit.

```
## Fitting of the distribution 'lnorm' by maximum likelihood
## Parameters :
##      estimate Std. Error
## meanlog 10.5565025 0.03662426
## sdlog    0.8502826 0.02589710
## Loglikelihood: -6367.344 AIC: 12738.69 BIC: 12747.27
## Correlation matrix:
##      meanlog sdlog
## meanlog      1      0
## sdlog         0      1
```

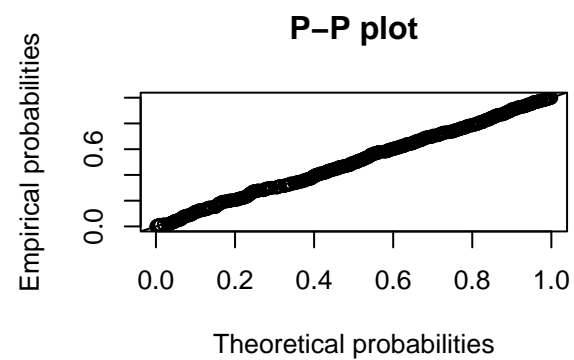
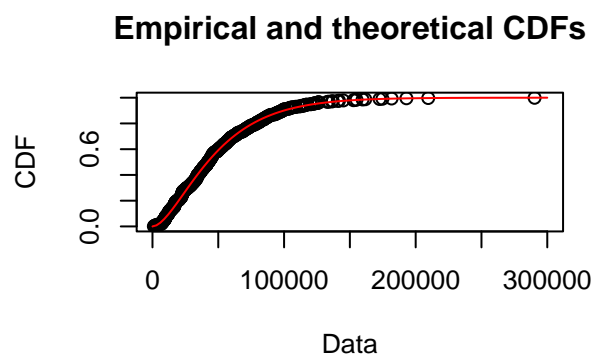
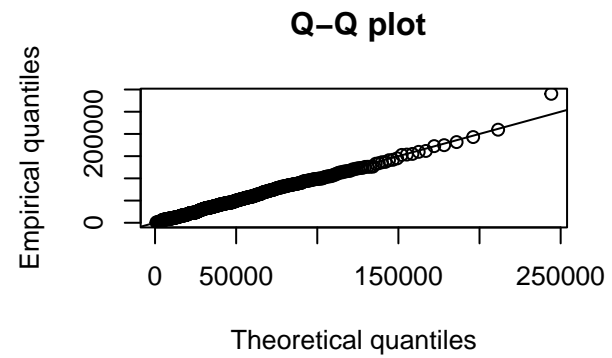
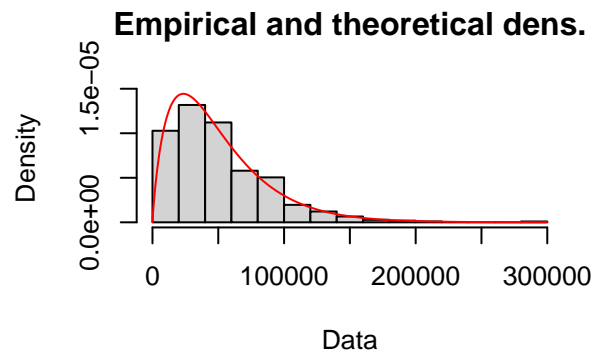


```
## Fitting of the distribution ' gamma ' by matching moments
## Parameters :
##      estimate
## shape 1.422273e+00
## rate  2.703472e-05
## Loglikelihood: -6362.27   AIC:  12728.54   BIC:  12737.12
```

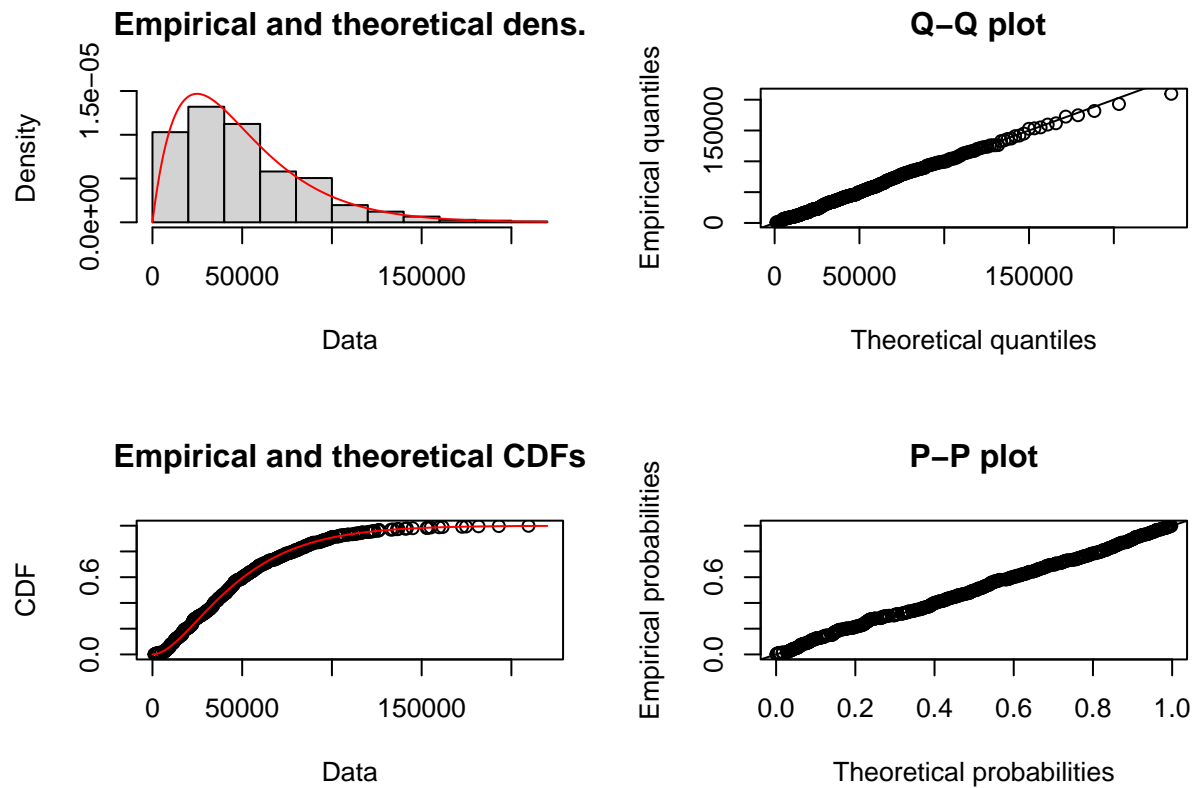


**Z-Score Outliers and 1 Remaining Point Removed** Note: So the largest outlier was a cortisol value of at least 300,000. I removed it and the gamma plot came out best (see below). When I removed the next largest outlier (cortisol < 250,000), all plots looked great, so I will further filter cortisol to all values below 250,000. AIC and BIC values also dropped substantially.

```
## Fitting of the distribution ' gamma ' by matching moments
## Parameters :
##      estimate
## shape 1.8665157270
## rate  0.0000369444
## Loglikelihood: -6276.834   AIC: 12557.67   BIC: 12566.23
```



```
## Fitting of the distribution ' gamma ' by matching moments
## Parameters :
##      estimate
## shape 1.986525e+00
## rate  3.967244e-05
## Loglikelihood: -6258.166   AIC:  12520.33   BIC:  12528.89
```

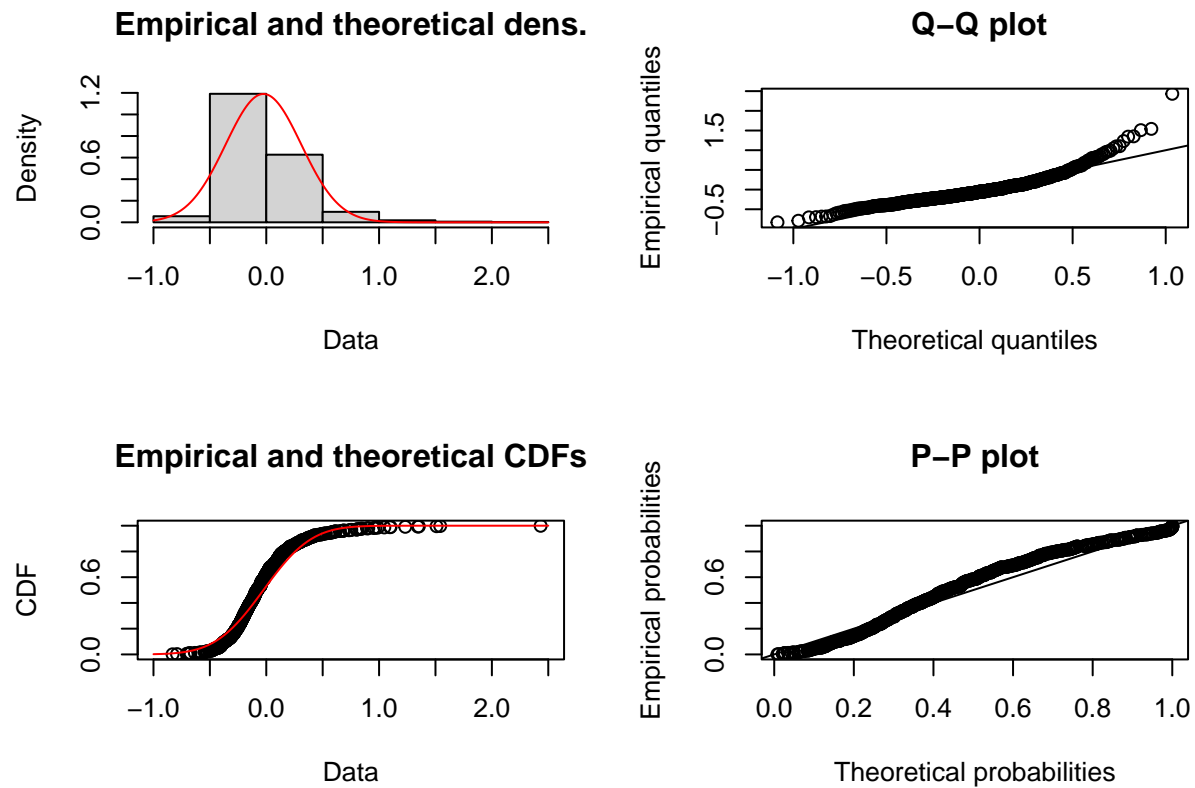


## ELBM

**Standard Inclusion Criteria** Note: Values must be positive to fit the lnorm, gamma, or exp distributions. Plots excluded.

```
## Fitting of the distribution ' norm ' by maximum likelihood
## Parameters :
##      estimate Std. Error
## mean -0.02503602 0.013275643
## sd    0.33532498 0.009386922
## Loglikelihood: -208.1688 AIC: 420.3376 BIC: 429.2543
## Correlation matrix:
##      mean sd
## mean  1  0
## sd    0  1
```

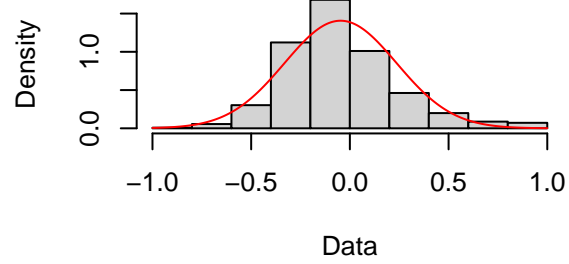




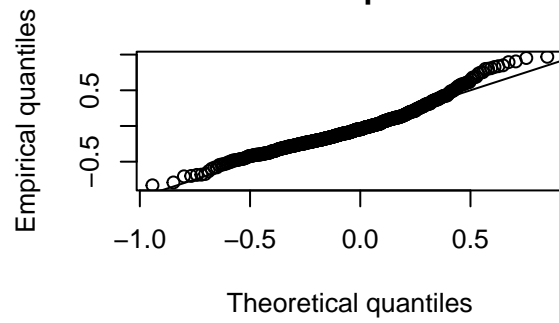
**Z-Score Outliers Removed** Note: When looking at the distribution without outliers, the normal distribution seems to fit decently well and is much improved compared to the normal distribution with the outliers kept in. I will fit with the normal distribution.

```
## Fitting of the distribution ' norm ' by maximum likelihood
## Parameters :
##      estimate Std. Error
## mean -0.04716648 0.011318374
## sd    0.28363764 0.008002851
## Loglikelihood: -99.77713 AIC: 203.5543 BIC: 212.4393
## Correlation matrix:
##      mean sd
## mean  1  0
## sd    0  1
```

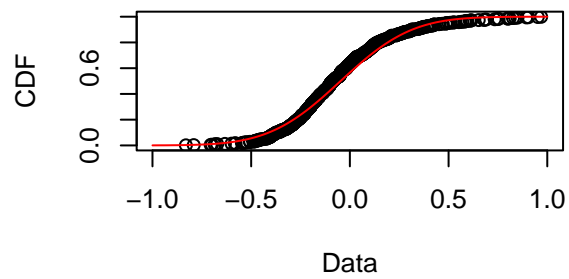
**Empirical and theoretical dens.**



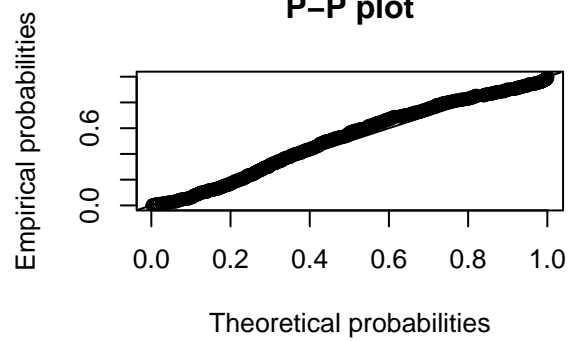
**Q-Q plot**



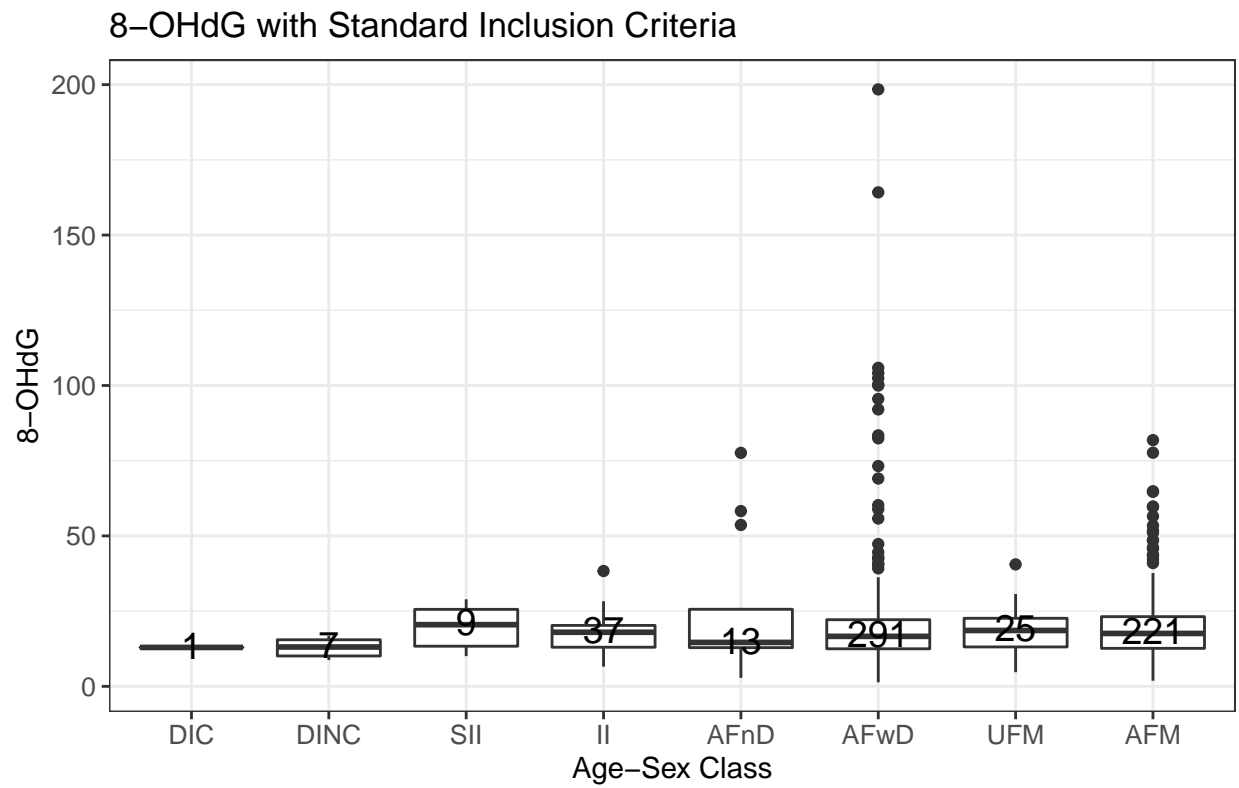
**Empirical and theoretical CDFs**



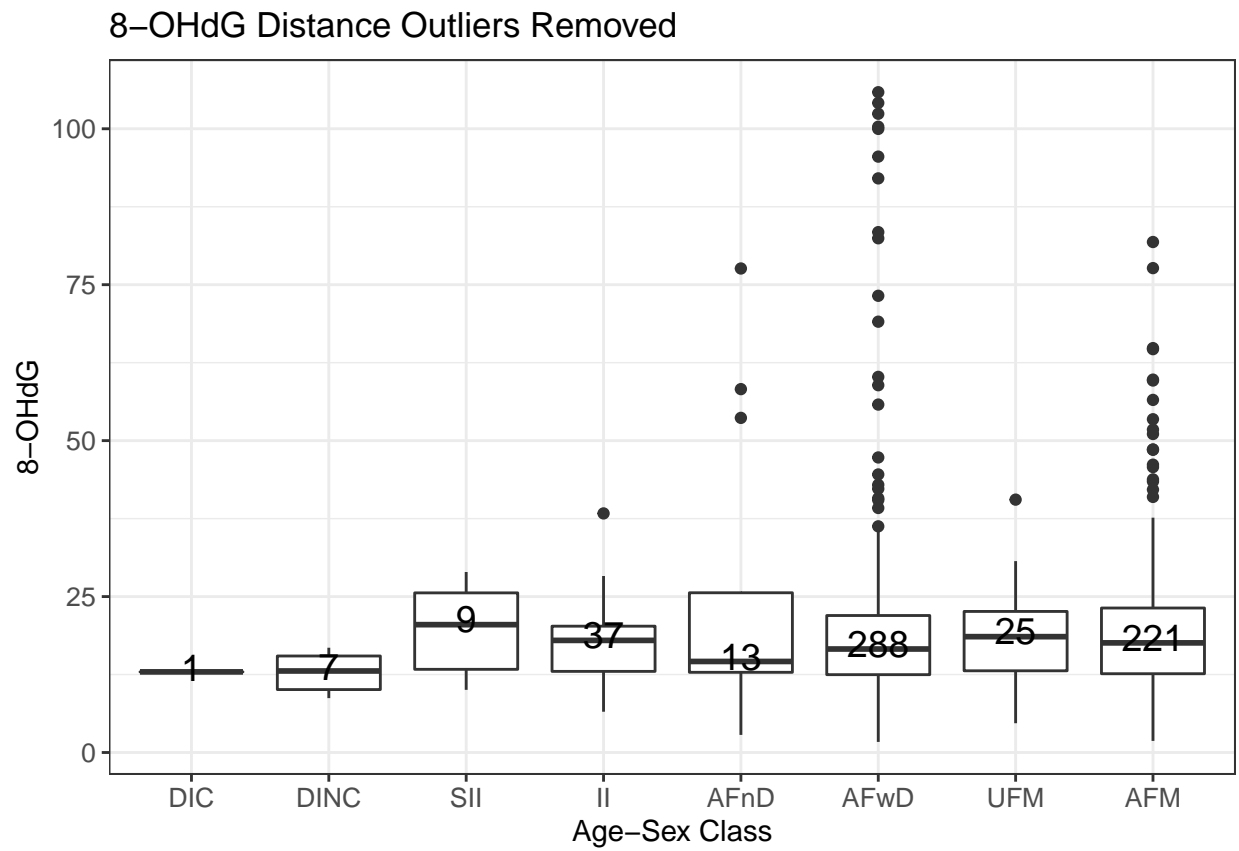
**P-P plot**

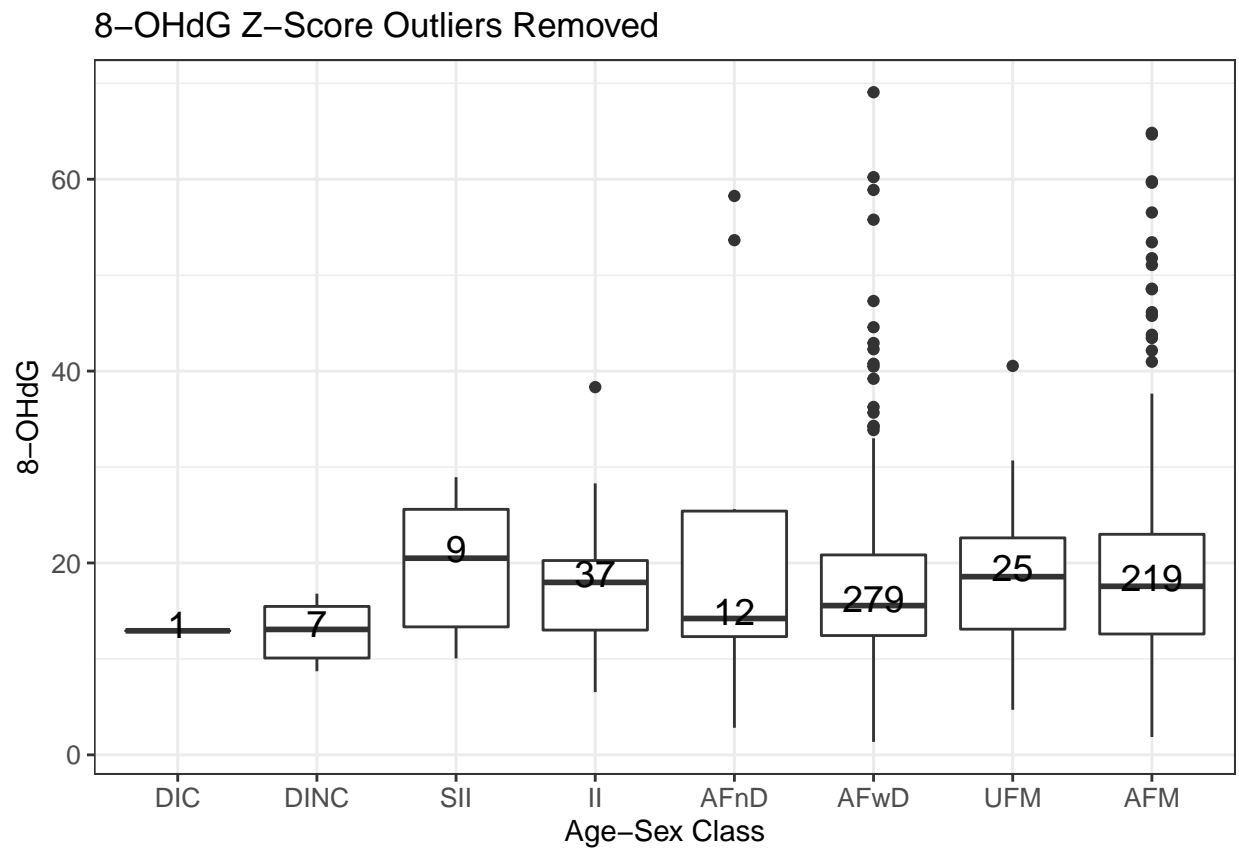


Box Plots of biomarkers by full age sex classes

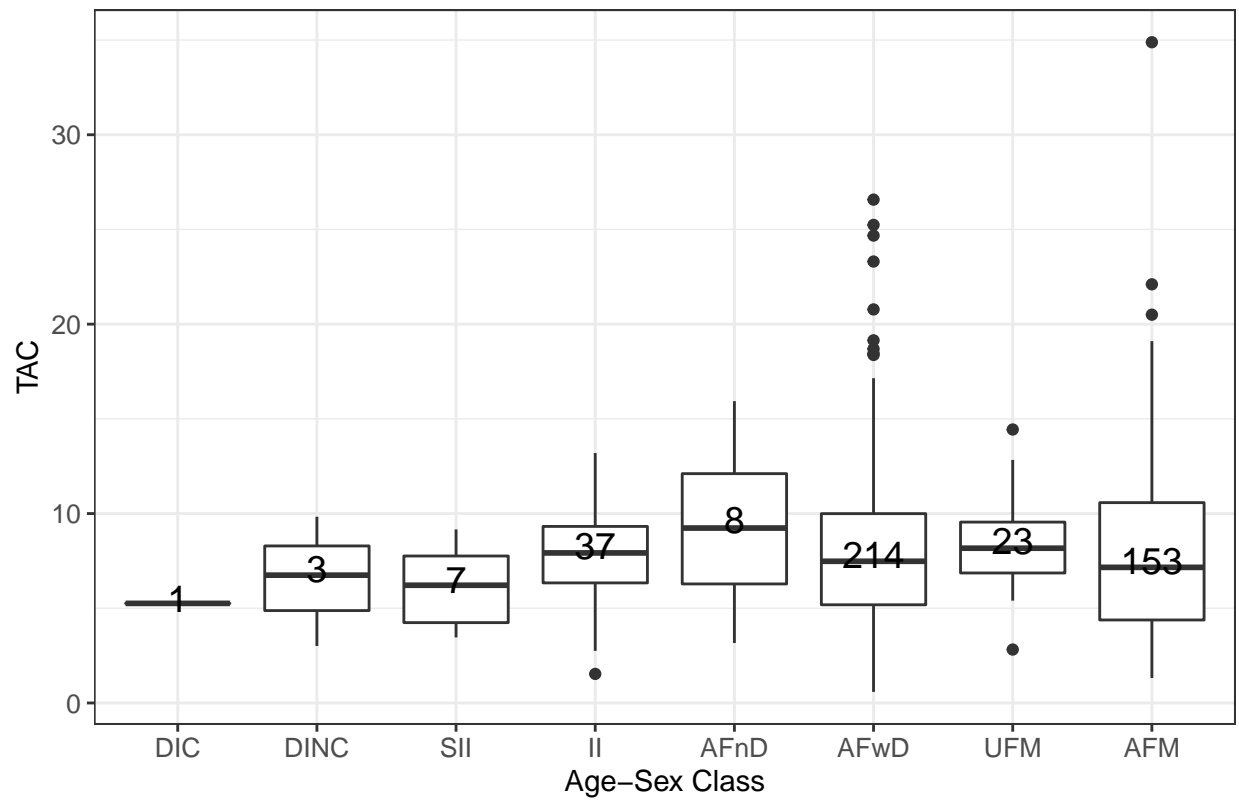


I cannot really identify a pattern aside from the chance for a lot of higher outliers in adult females

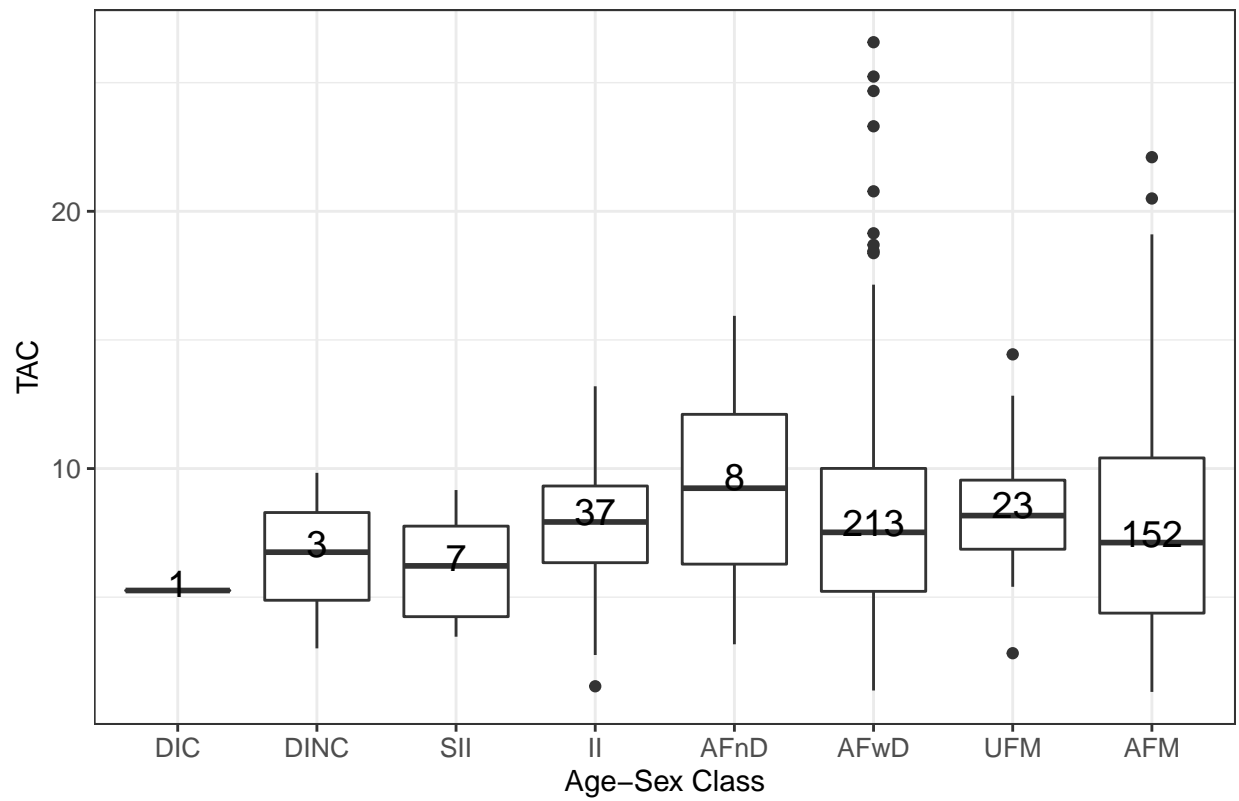




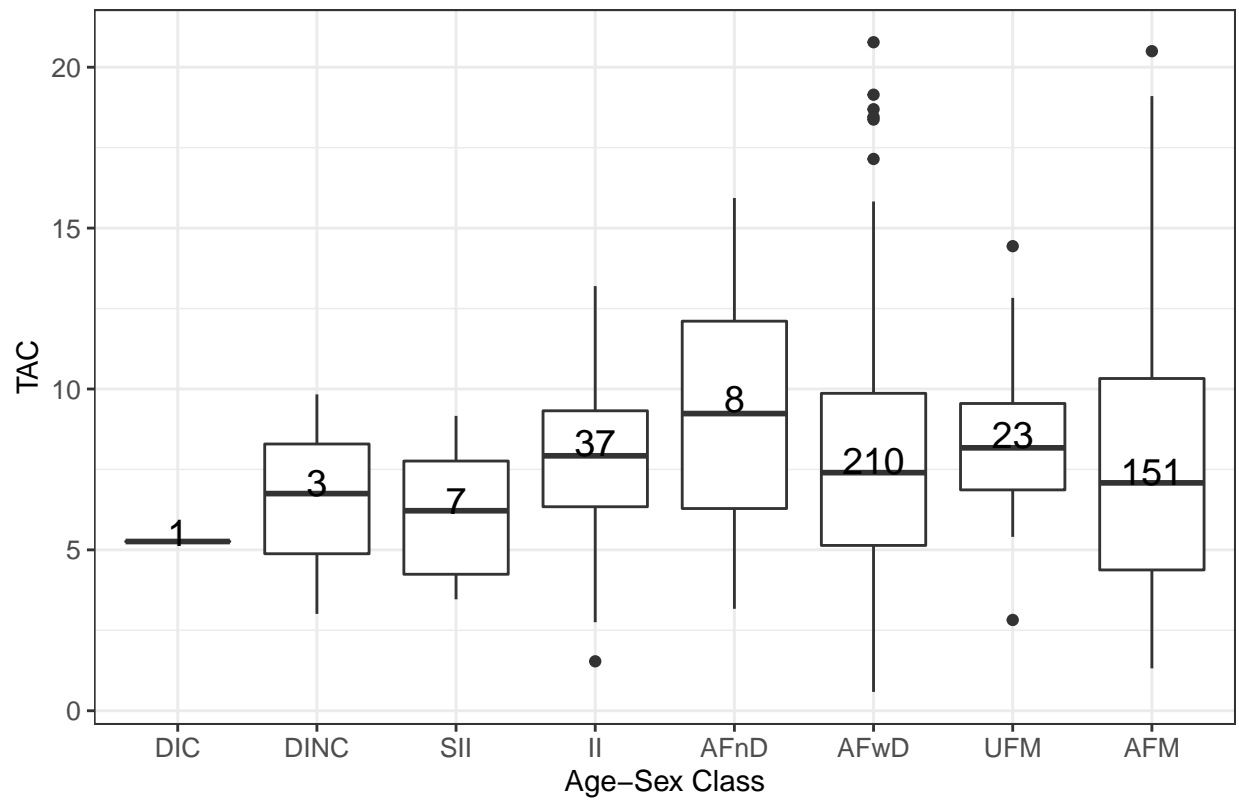
TAC with Standard Inclusion Criteria



TAC Distance Outliers Removed

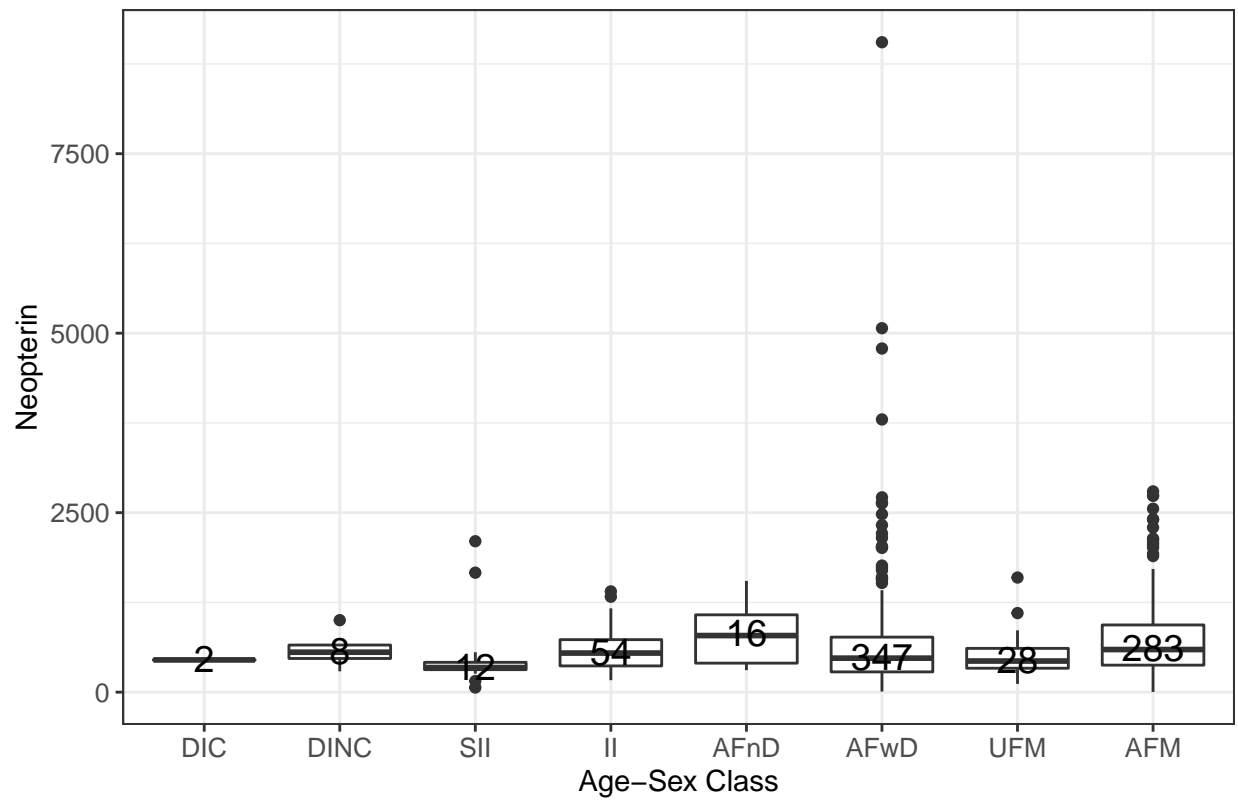


TAC Z-Score Outliers Removed

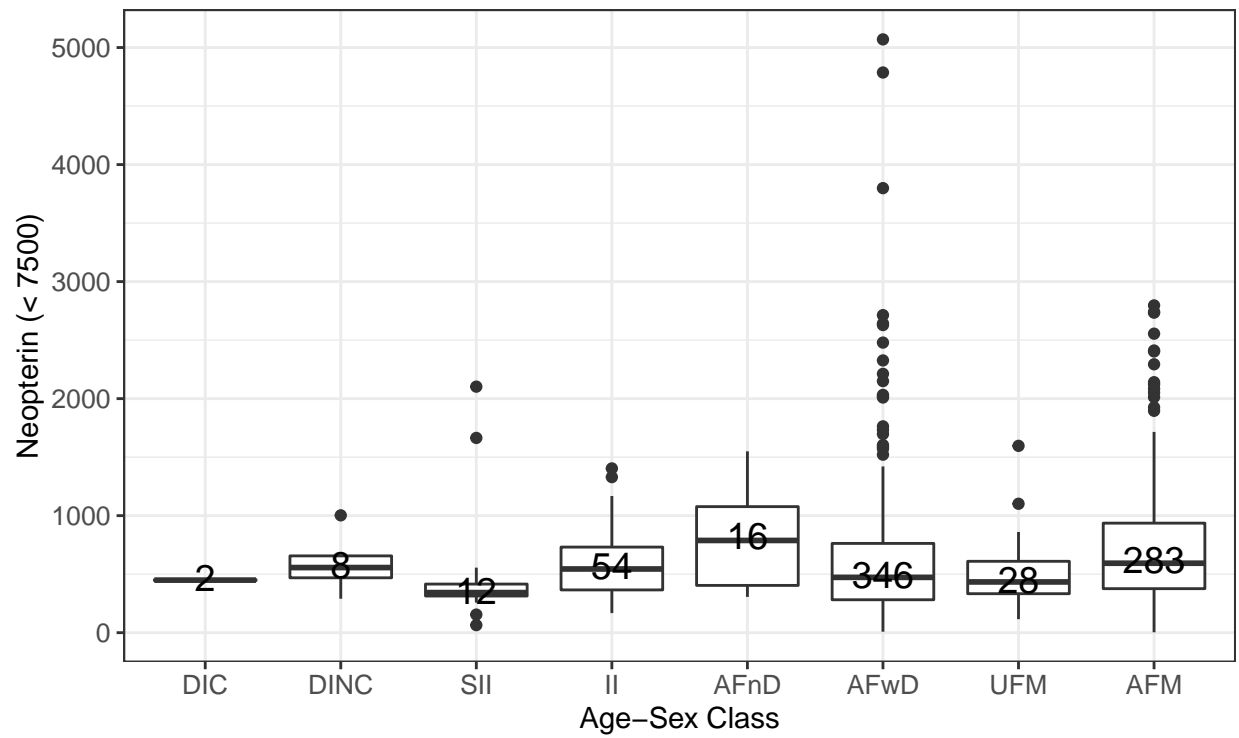




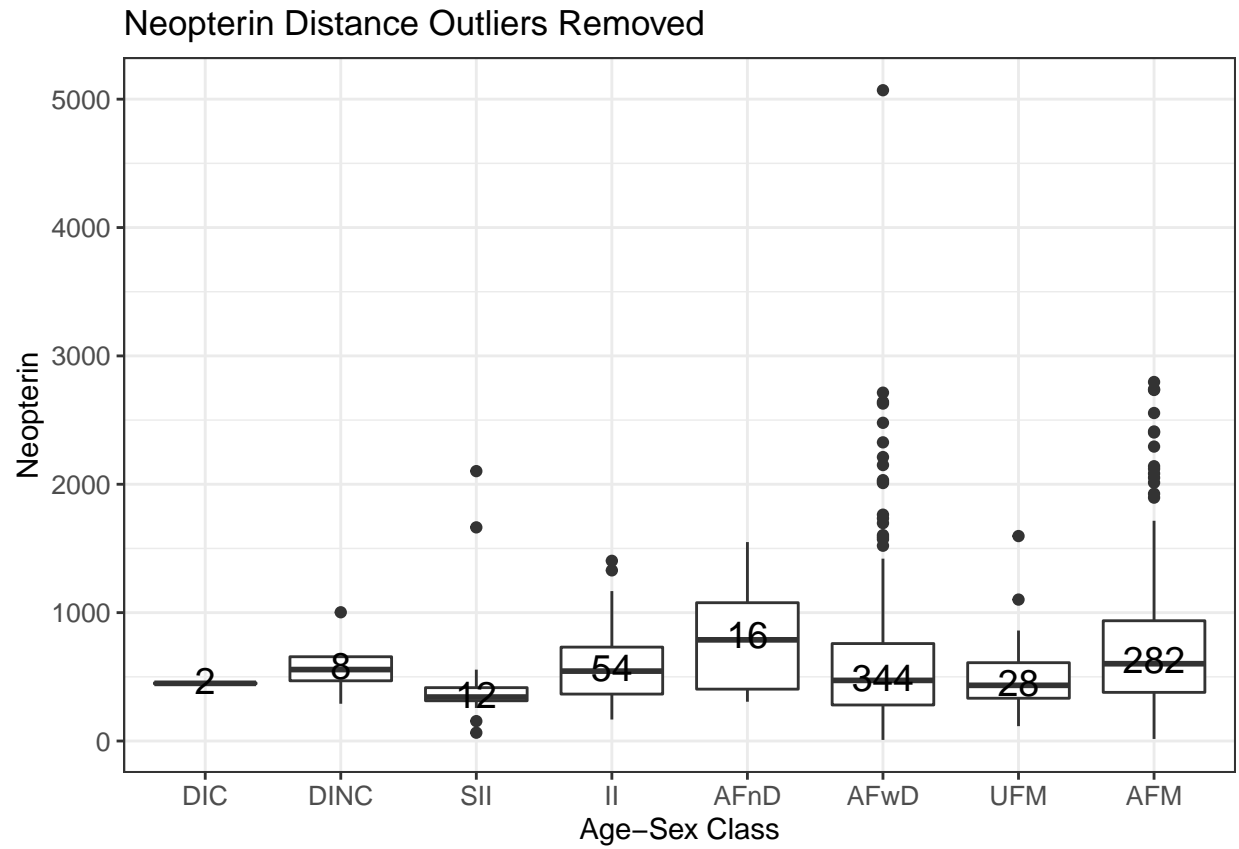
Neopterin with Standard Inclusion Criteria

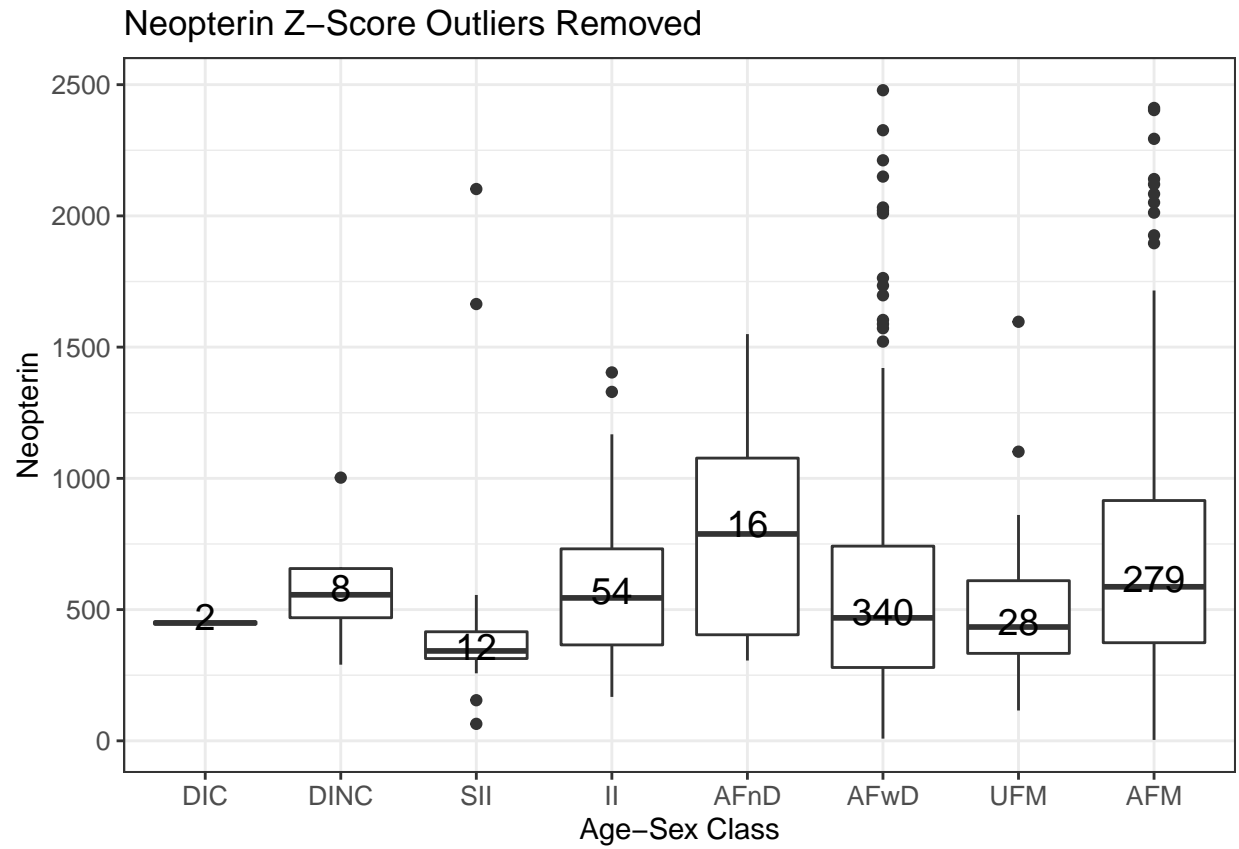


Neopterin with Single High Outlier Removed

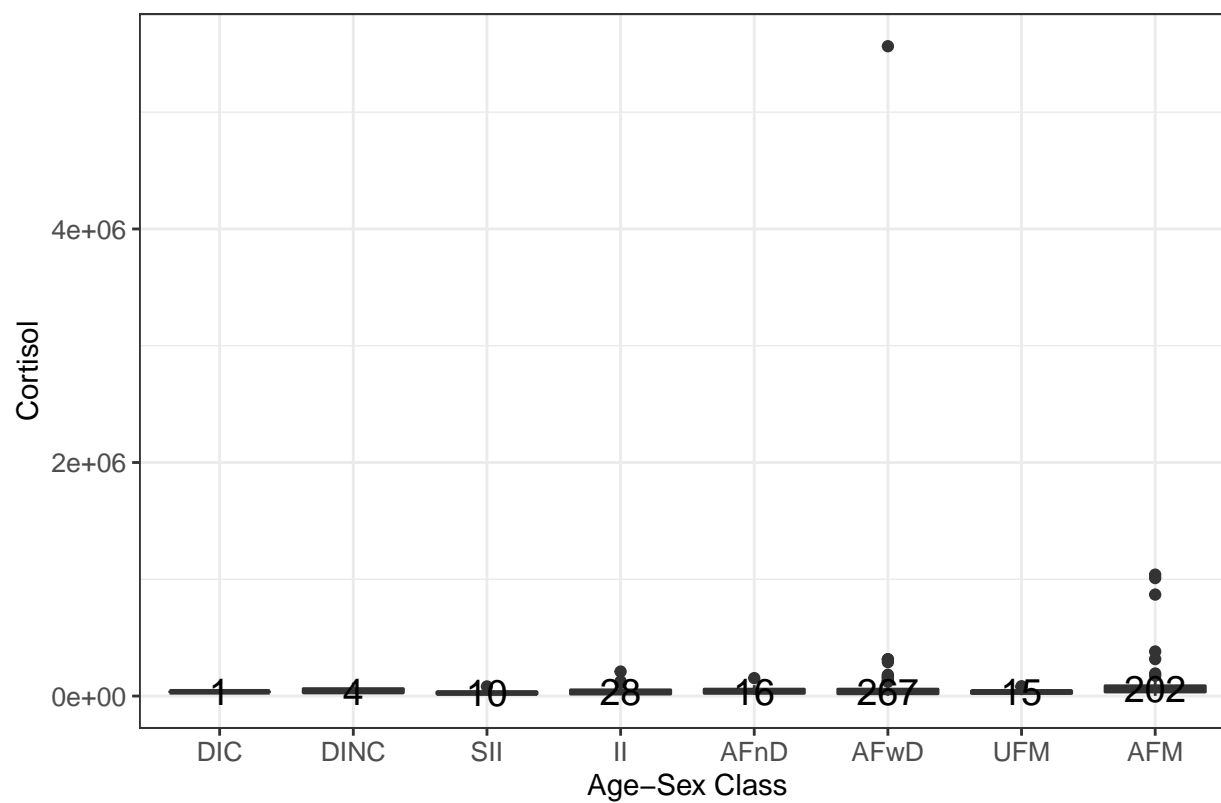


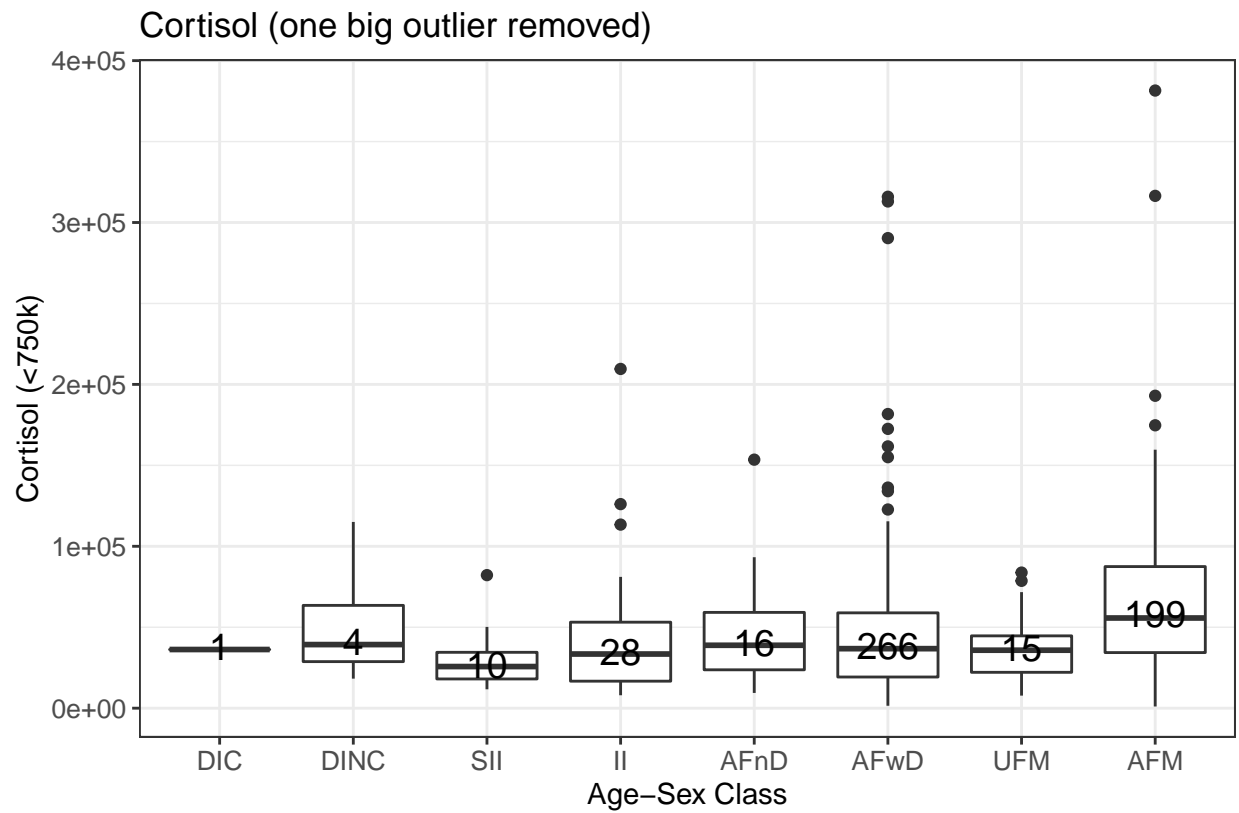
It does appear that adult flanged males higher median levels of neopterin





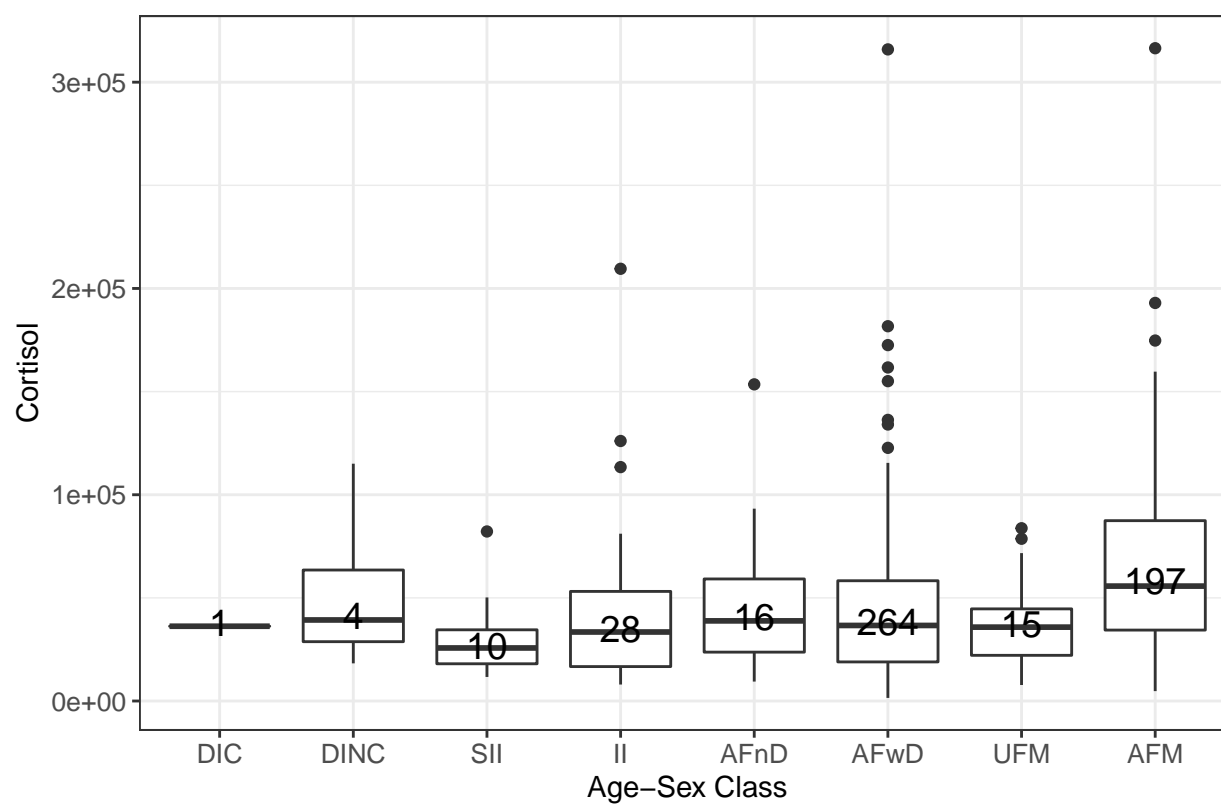
Cortisol with Standard Inclusion Criteria

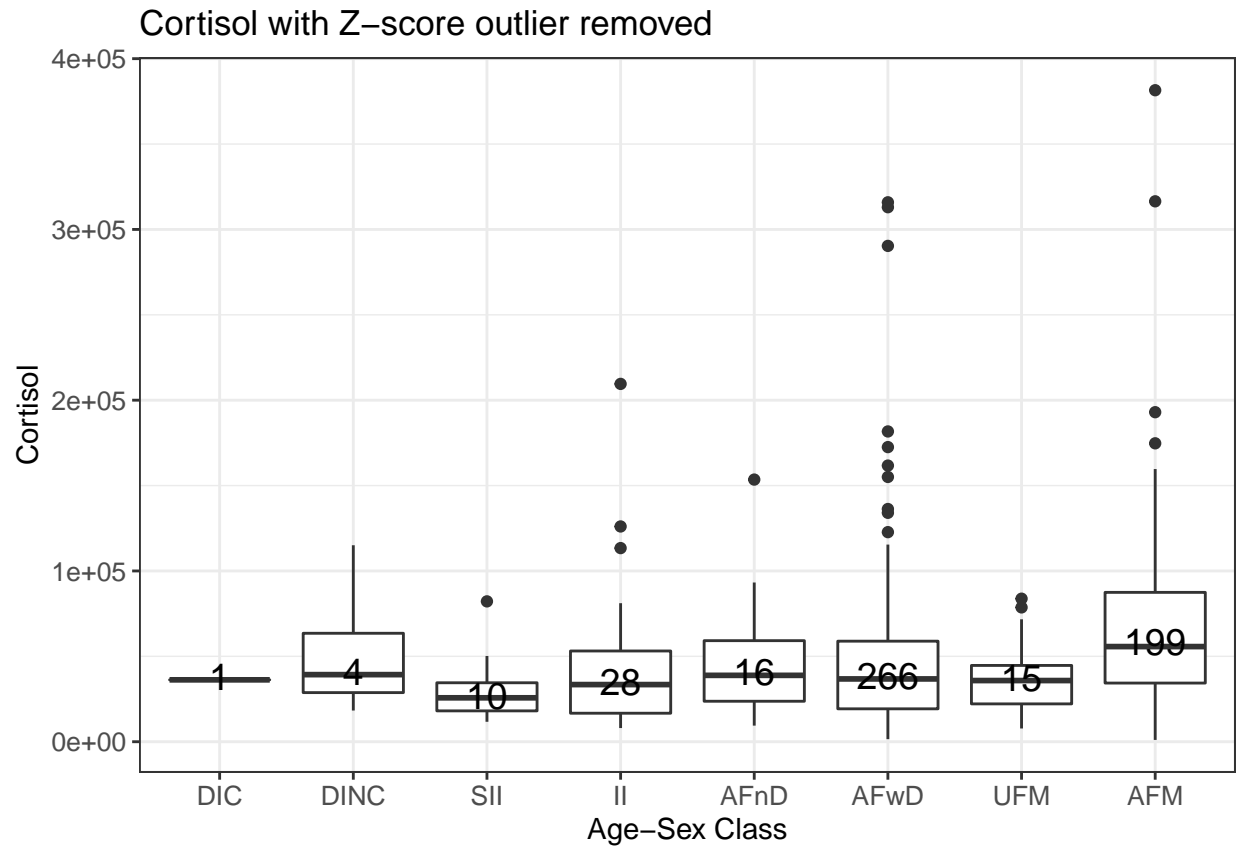




Flanged Males appear to have higher levels of cortisol

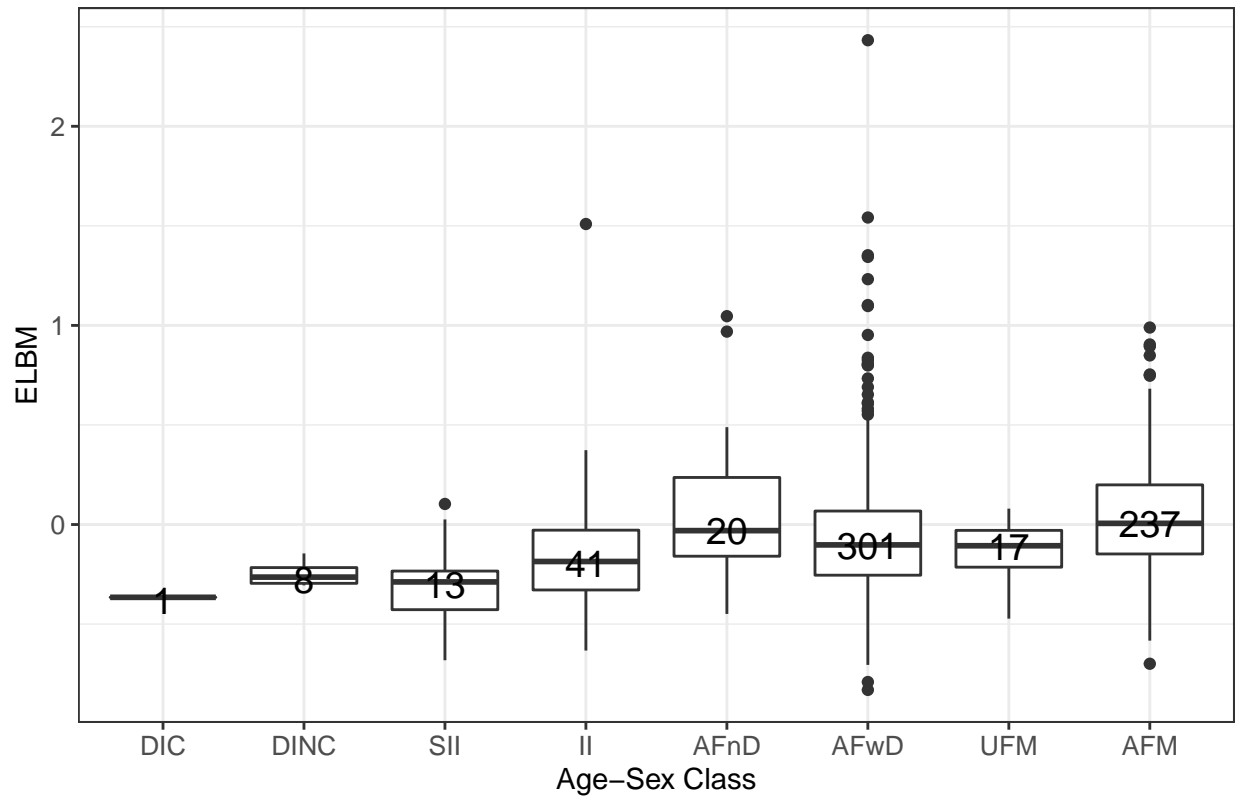
Cortisol with Distance outliers Removed

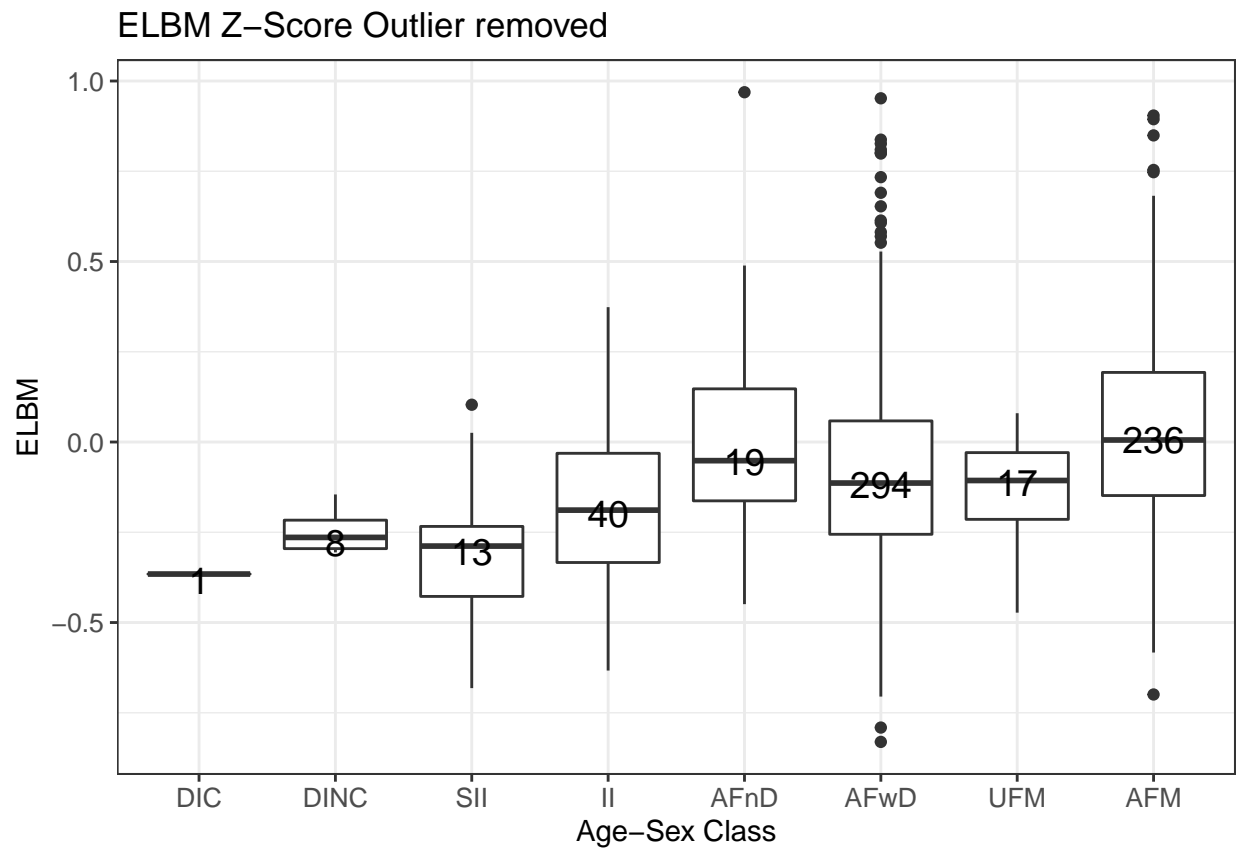






ELBM Standard Inclusion Criteria

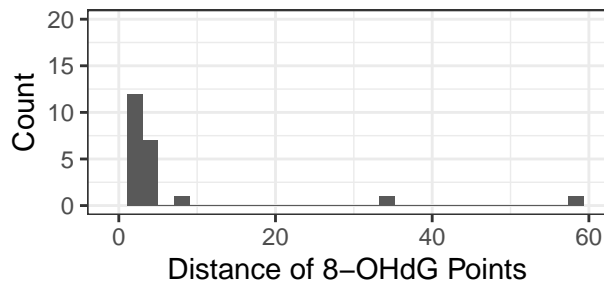




Work on Distance Outlier Detection

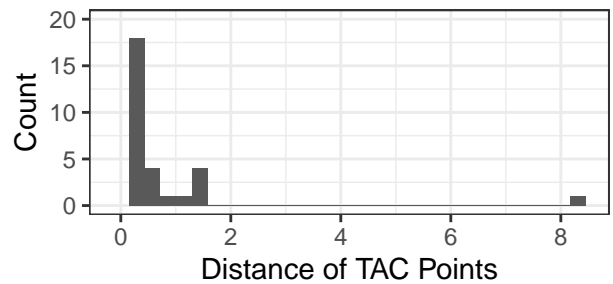
Distribution of Successive  
8-OHdG Distances

y-axis limited to 20



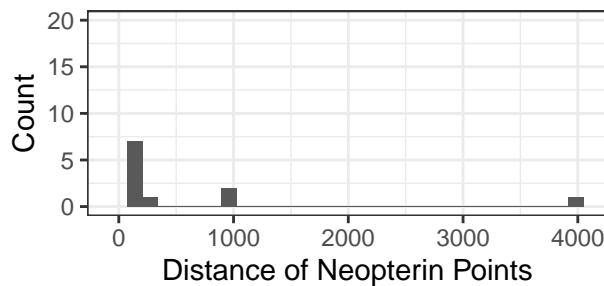
Distribution of Successive  
TAC Distances

y-axis limited to 20



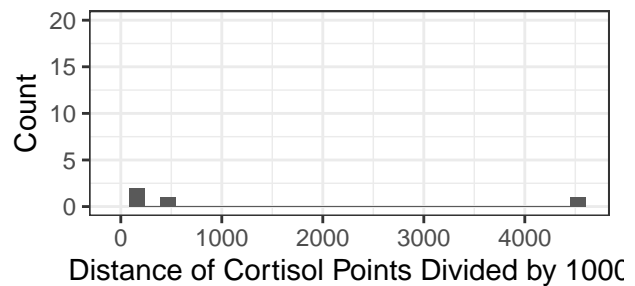
Distribution of Successive  
Neopterin Distances

y-axis limited to 20



Distribution of Successive  
Cortisol Distances

y-axis limited to 20

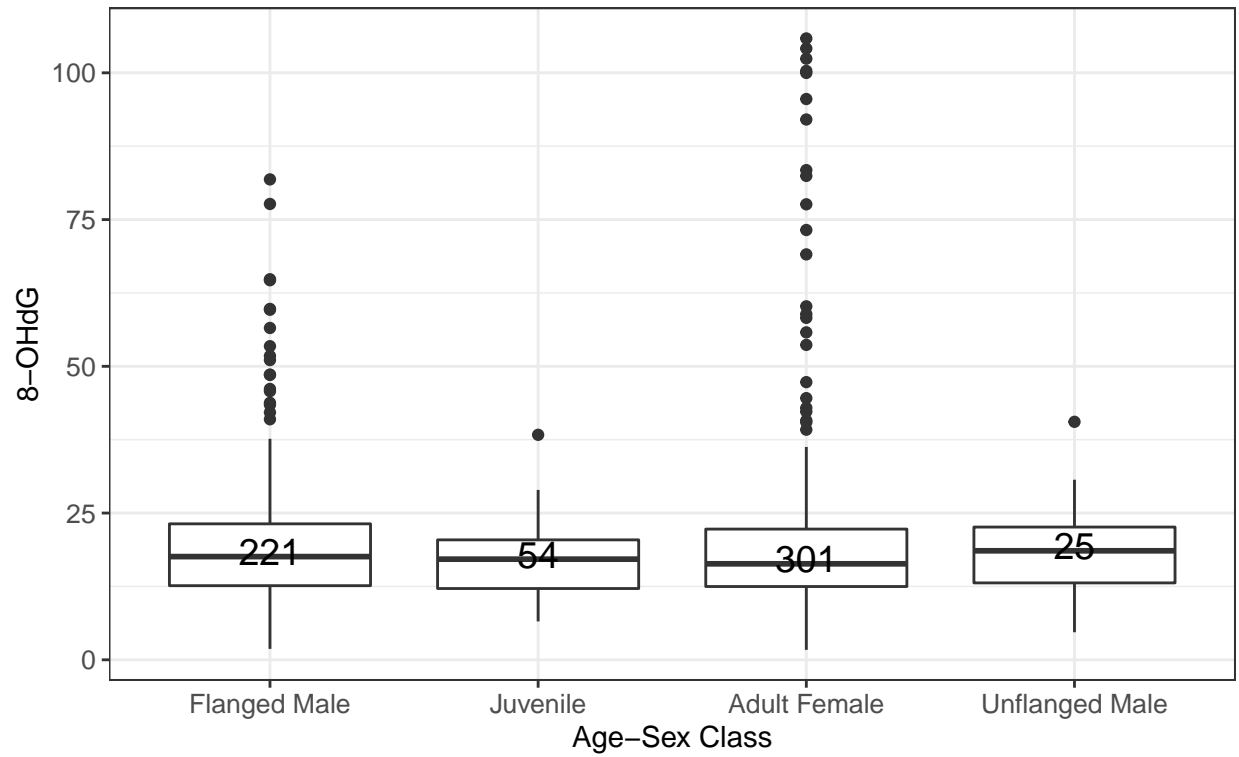


Based on these plots, I am going to flag the distance outliers as follows: \* 8-OHdG: distances greater than 20 \* TAC: distances greater than 1.5 \* Neopterin: Distances greater than 500 \* Cortisol: distance greater than 80,000 (800 on the plot)

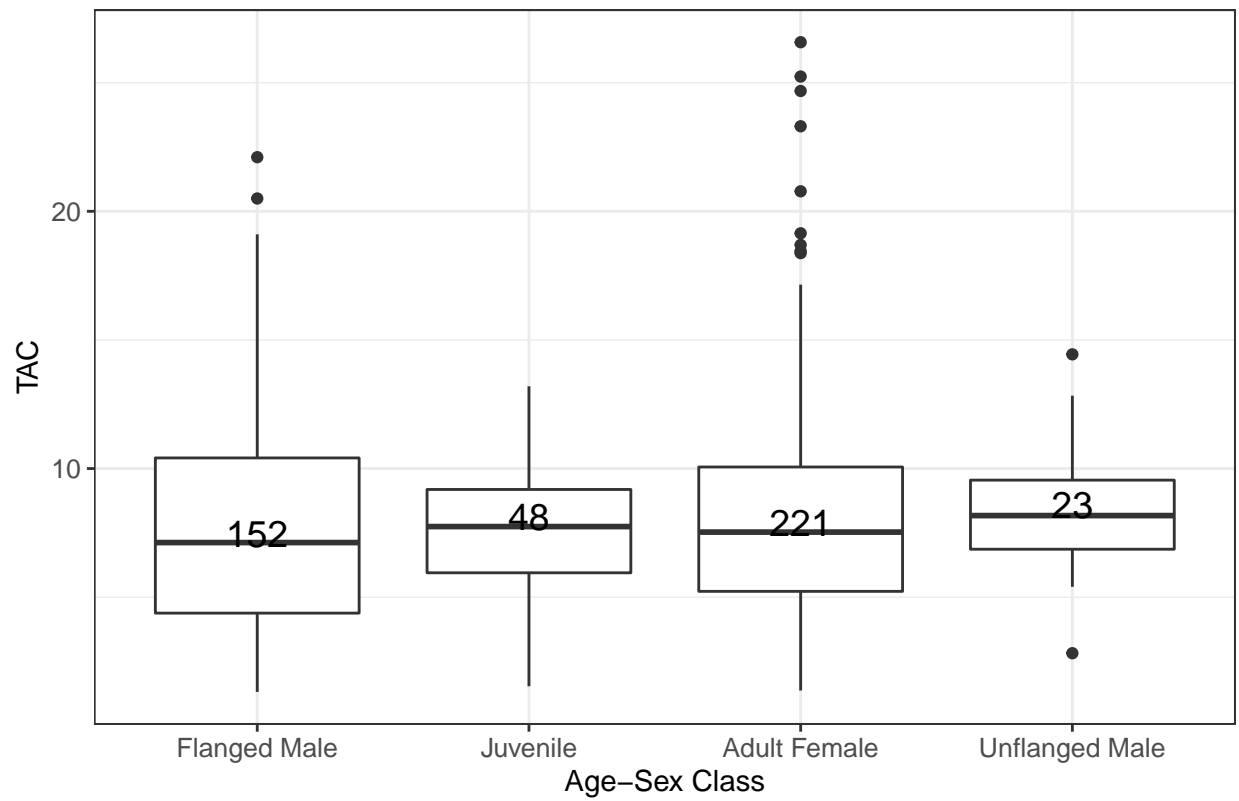
*Note: I went with the distance metric and that resulted in some of the distributions in the supplement of this document where I tried to determine the best link function for later GAMM fittings. There still appears to be some outliers, so perhaps I need to use the Z-score or 1.5 x IQR approach*

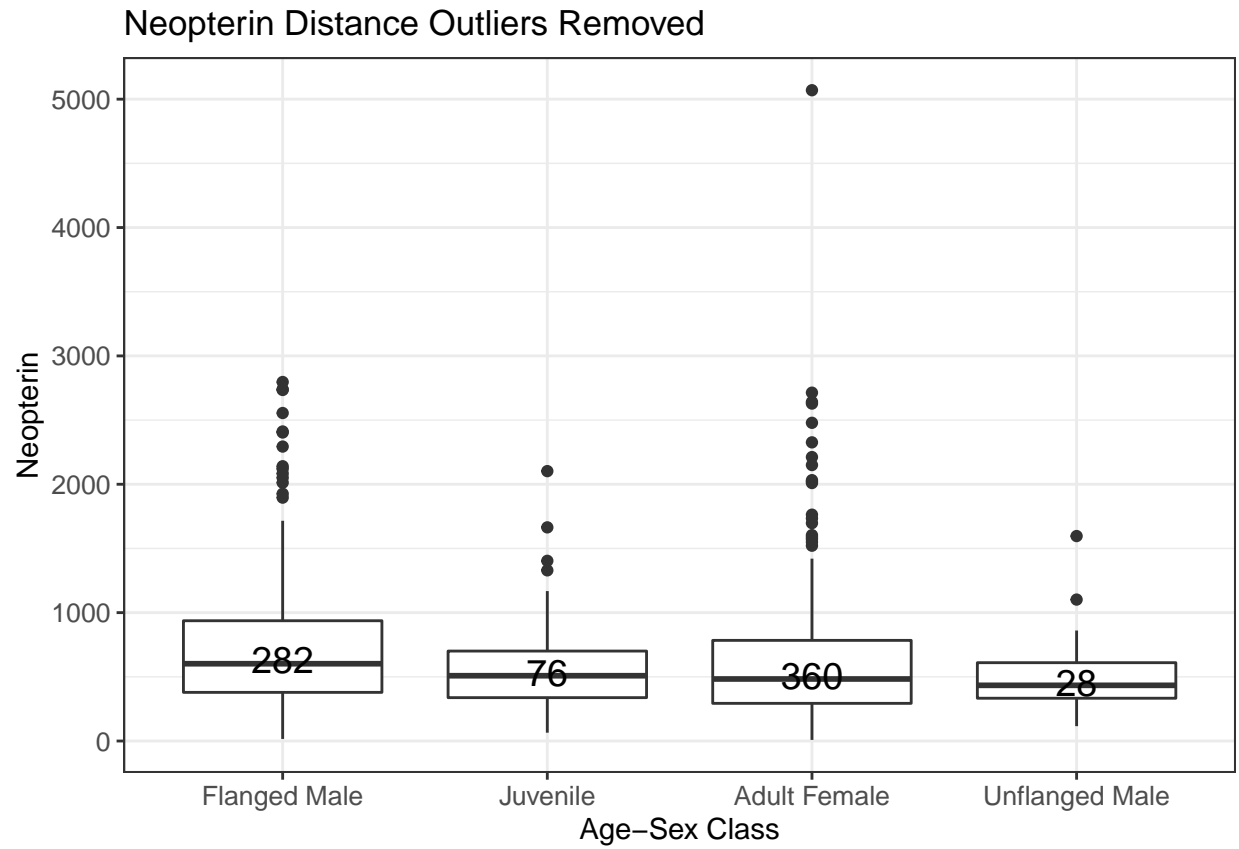
## 8-OHdG Distance Outliers Removed

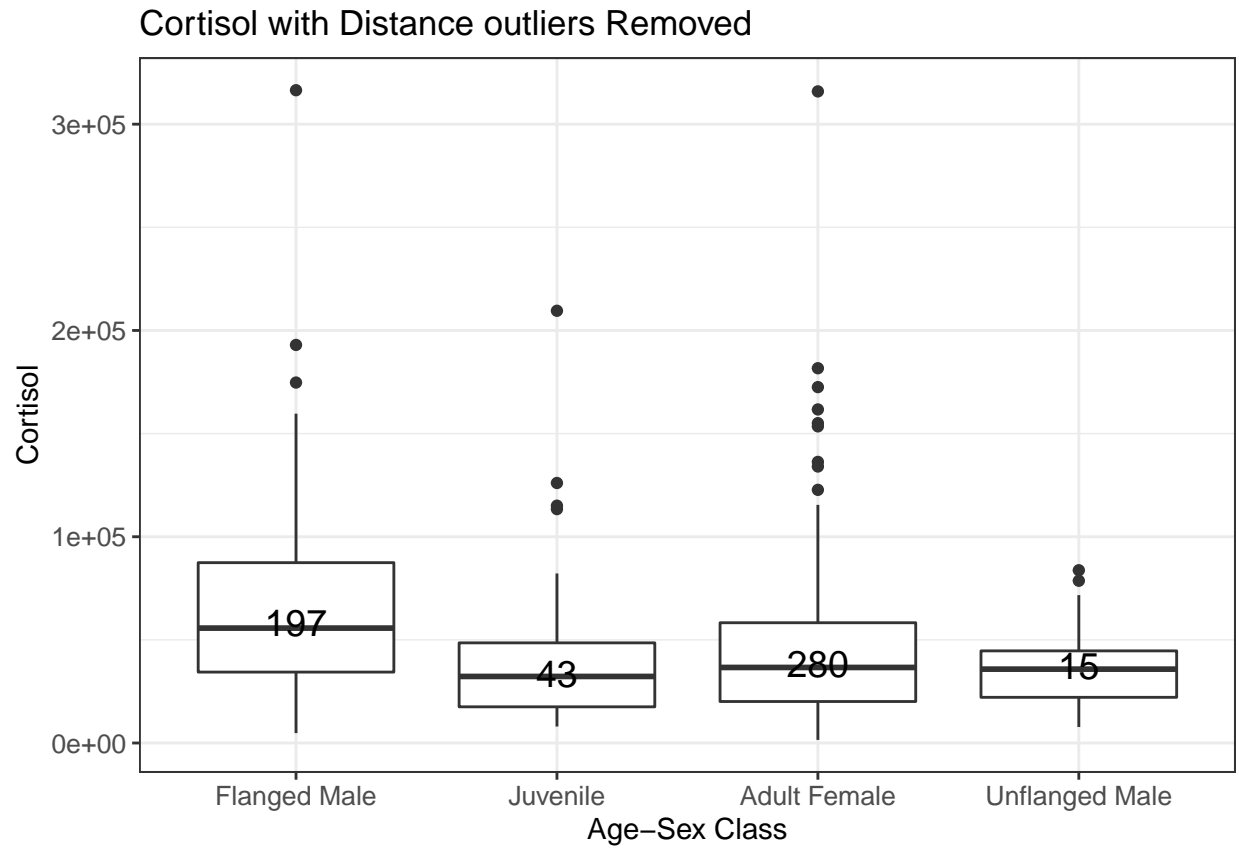
Truncated Age-Sex Classes



TAC Distance Outliers Removed

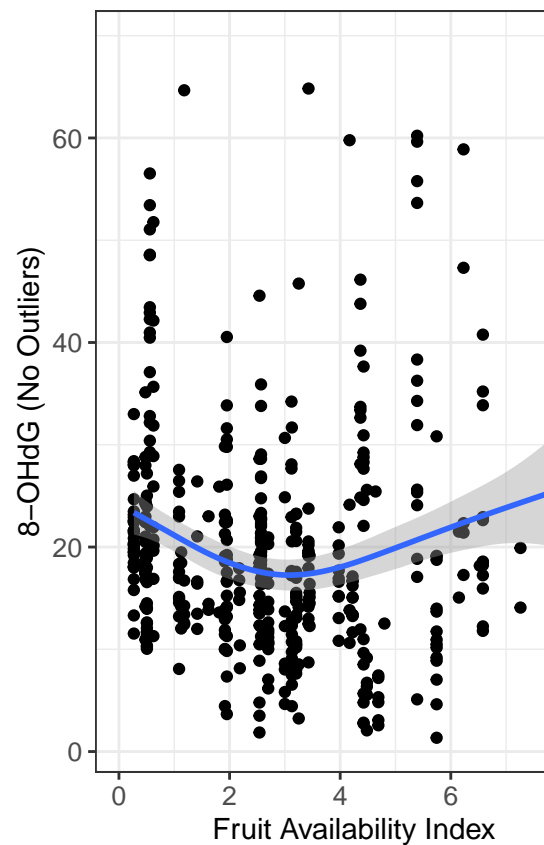
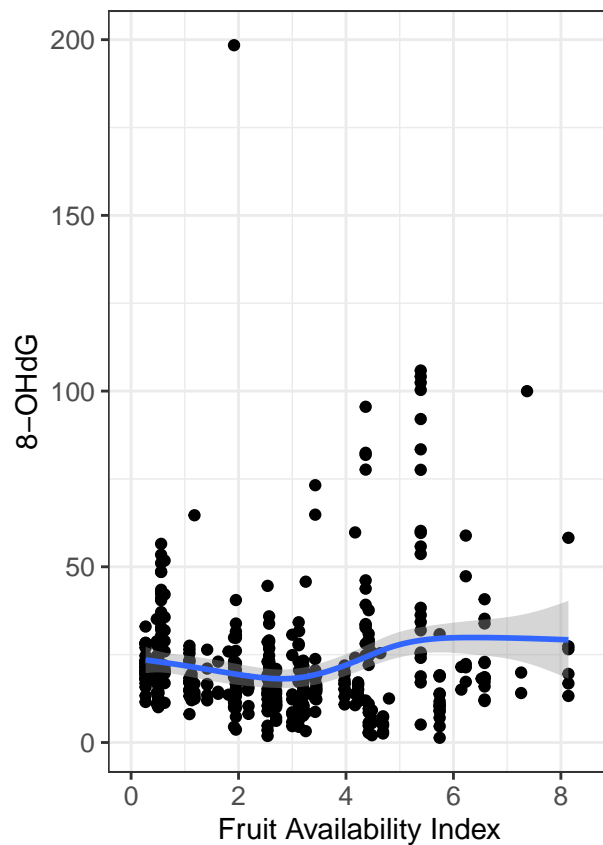




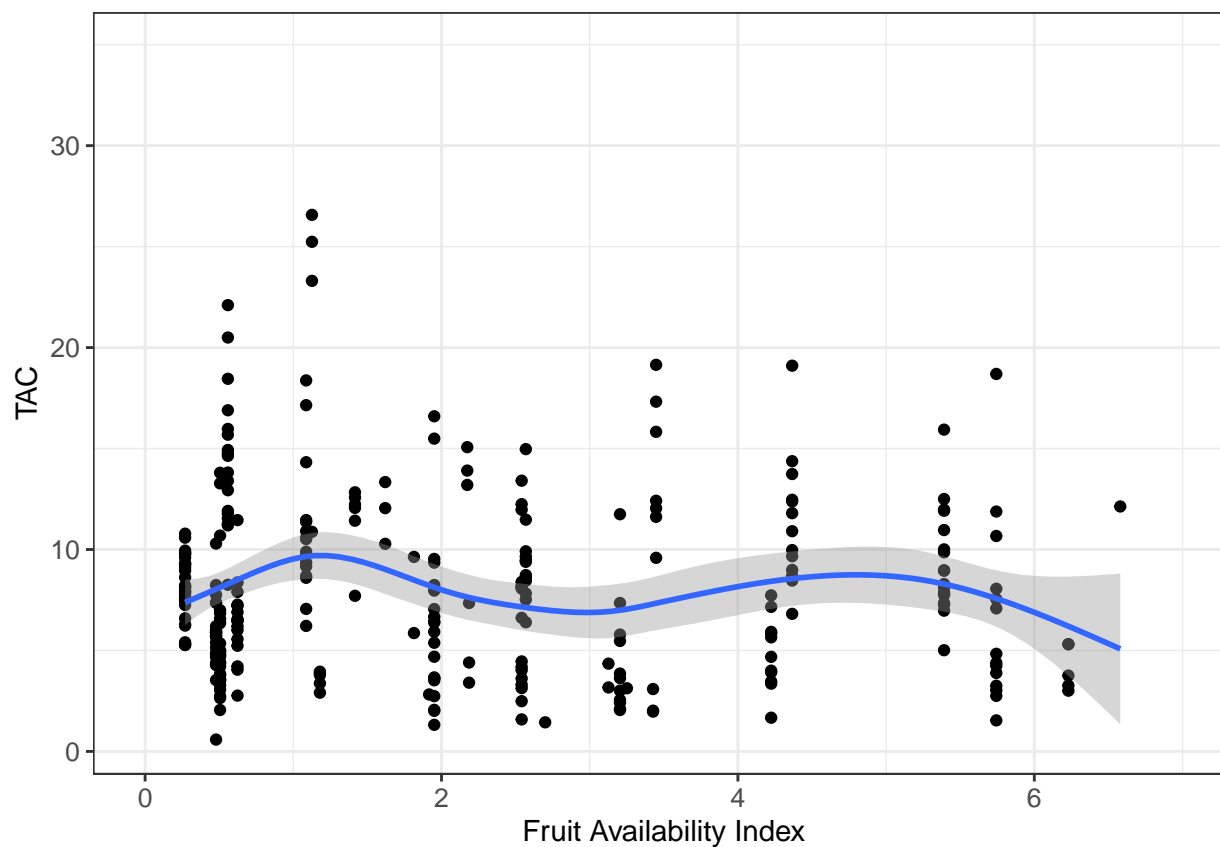




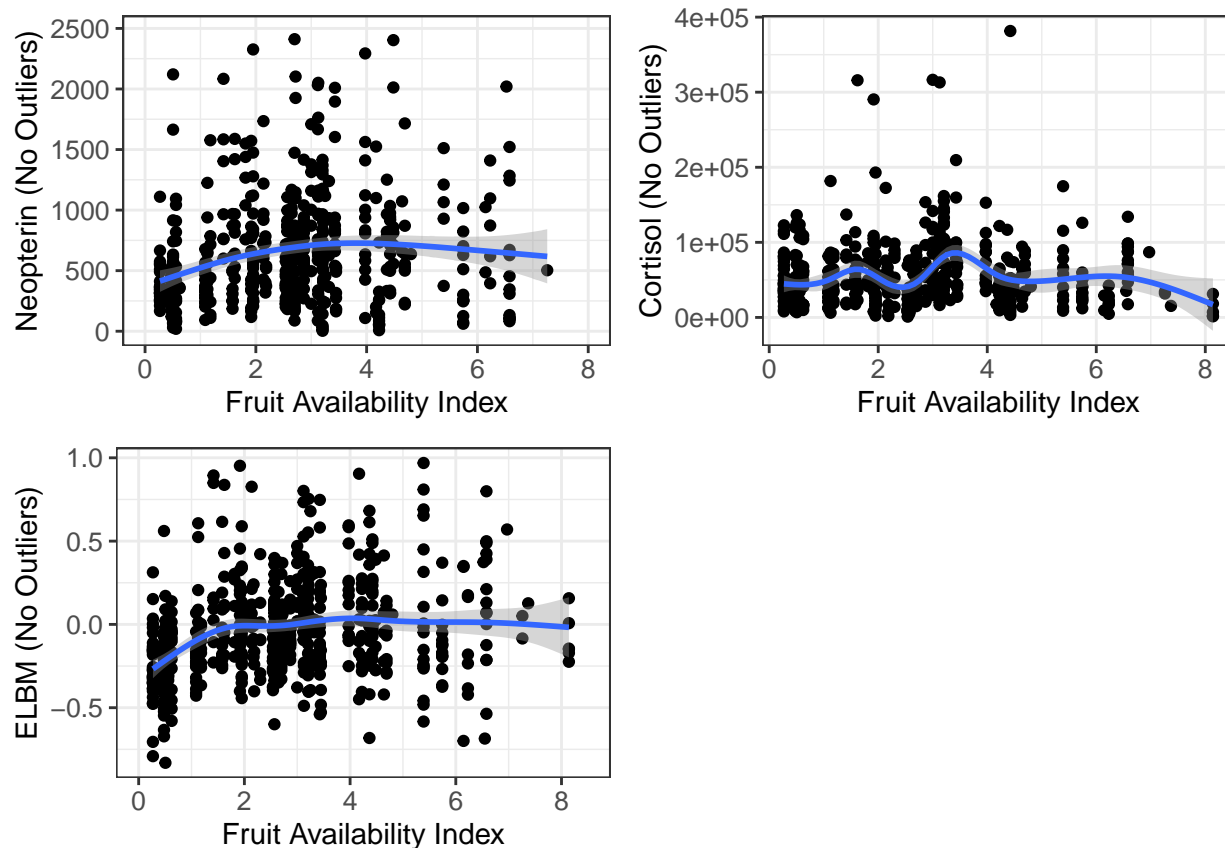




Biomarkers by FAI



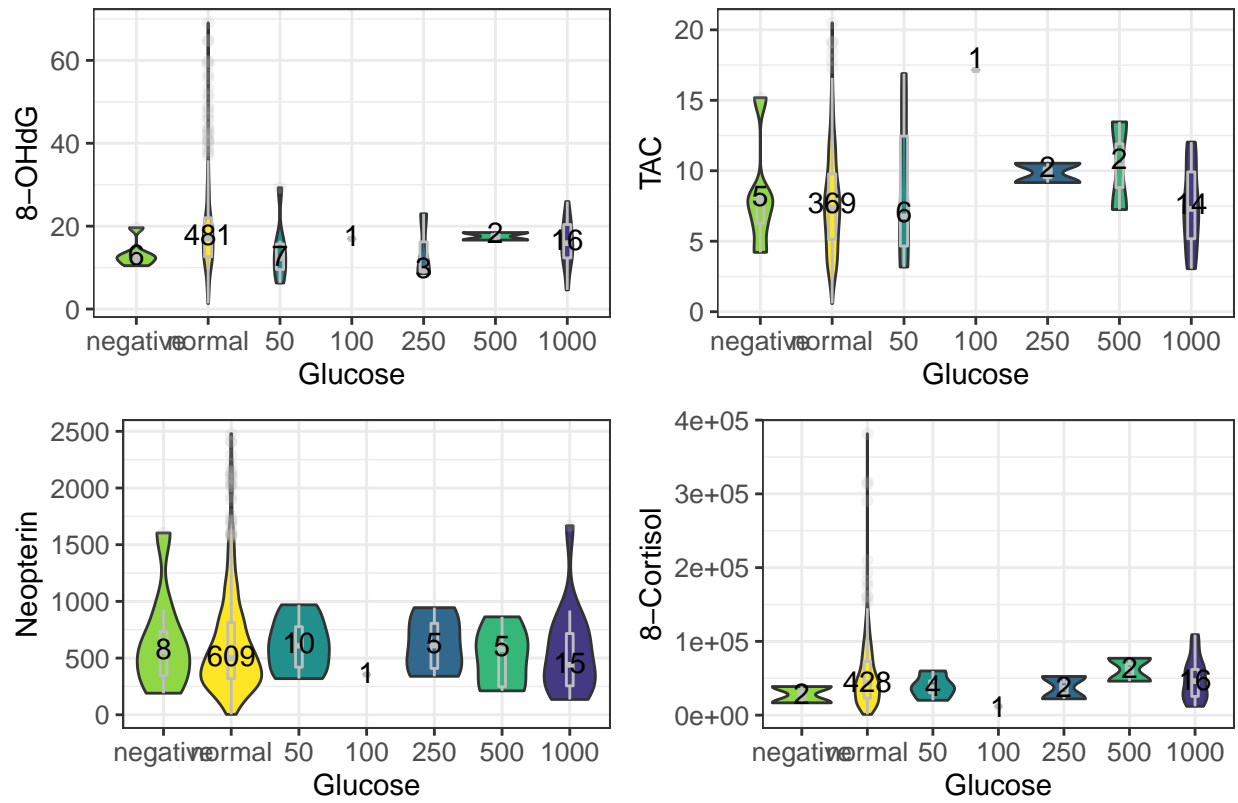
The increase in 8-OHdG related to FAI seems to be driven by a single point. Note that a much higher outlier exists elsewhere on the lower end of FAI so I doubt FAI is actually related to this at all. If I remove these two outliers, it would appear, ever so slightly, that there are two plateaus, where it starts out lower and then increases. However, this is not really suggestive of anything; FAI is not capturing information relevant to oxidative stress. TAC definitely does not seem to be associated with it. I mean maybe there is a bit of a bimodal peak. Maybme.



Both neopterin and ELBM seem to increase with FAI up to a point and then become stable; there is a rather wide spread to the data. This increase runs from 0 to 2% FAI, where caloric intake is most likely at it's lowest and when orangutans might be undernourished. While an unsubstantiated statement, it makes sense that these relationships would be stable except in the extreme. The relationship between cortisol and FAI is almost nonsensical; further analysis has been disregarded. Conclusive statements will require modelling that accounts for individual variation.

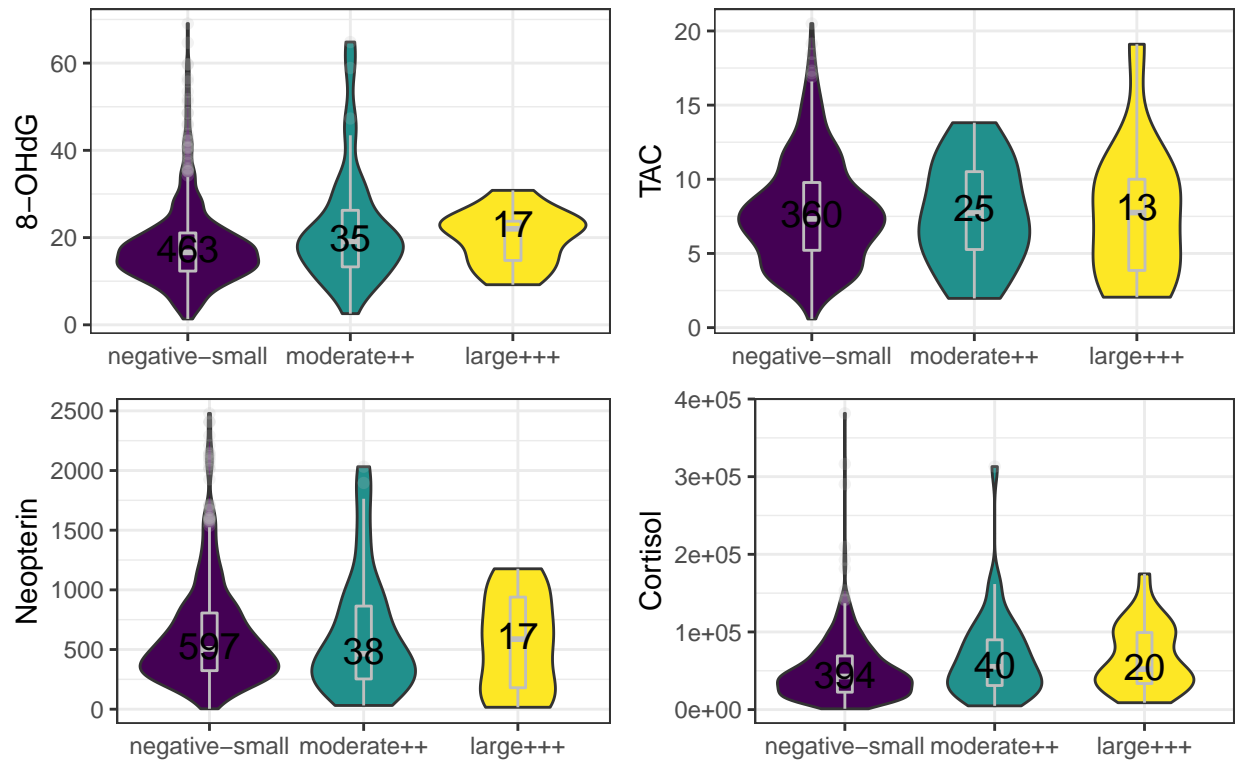
**Biomarkers by Select Forest Chemstrip Data** This part of the report was inspired by a paper talking about the effects of high glucose induced increases in oxidative stress episodes on myogenesis, particularly acceleration (Liu et al. 2020).

## Biomarkers and Glucose



Given the tiny sample sizes with a glucose reading above normal, I doubt these distributions have any true relevance.

## Biomarkers and Ketone Bodies



## Ketone Bodies

Based solely on violin plots, it looks like high levels of ketones *might* increase oxidative stress.

```
##
## Maximum number of PQL iterations: 20

##
## Maximum number of PQL iterations: 20

##
## Maximum number of PQL iterations: 20

##
## Maximum number of PQL iterations: 20

##
## Family: gaussian
## Link function: log
##
## Formula:
## ohdg_include ~ forest_ketones
##
## Parametric coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)    2.97642    0.04524  65.796 < 2e-16 ***
```

```

## forest_ketonesmoderate++ 0.28884    0.10613    2.722 0.00672 **
## forest_ketoneslarge+++   0.05053    0.19226    0.263 0.79280
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
## R-sq.(adj) = 0.00519
##   Scale est. = 228.16    n = 525

##
## Family: Gamma
## Link function: inverse
##
## Formula:
## tac_include ~ forest_ketones
##
## Parametric coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.125656   0.004623  27.181  <2e-16 ***
## forest_ketonesmoderate++ 0.006373   0.013727   0.464   0.643
## forest_ketoneslarge+++  0.003278   0.018773   0.175   0.861
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
## R-sq.(adj) = -0.00439
##   Scale est. = 0.24007    n = 403

##
## Family: gaussian
## Link function: log
##
## Formula:
## tac_include ~ forest_ketones
##
## Parametric coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)    2.08882    0.03796  55.028  <2e-16 ***
## forest_ketonesmoderate++ -0.05012    0.11131  -0.450   0.653
## forest_ketoneslarge+++  -0.01018    0.15004  -0.068   0.946
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
## R-sq.(adj) = -0.00448
##   Scale est. = 16.151    n = 403

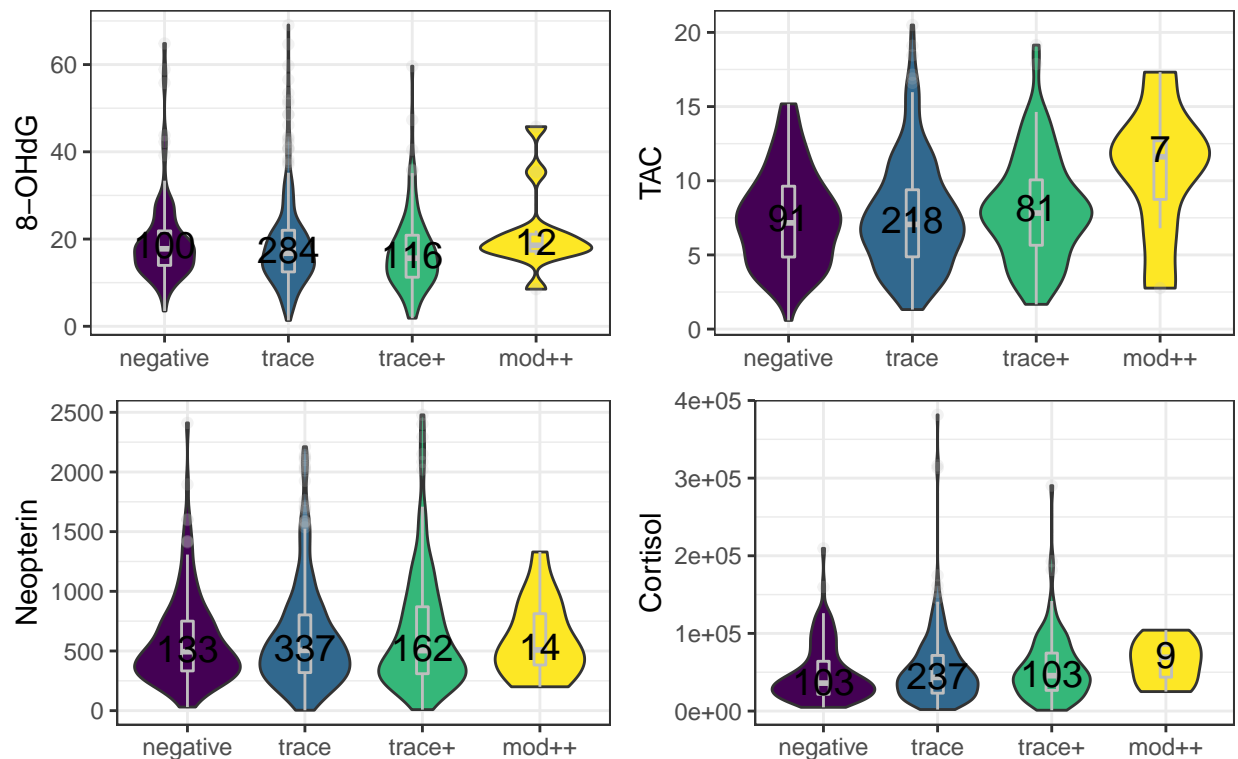
##
## Family: Gamma
## Link function: inverse
##
## Formula:
## cort_include ~ forest_ketones

```

```
##
## Parametric coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    2.012e-05  1.090e-06  18.465  <2e-16 ***
## forest_ketonesmoderate++ -3.767e-06  1.926e-06  -1.956  0.0511 .
## forest_ketoneslarge+++  -4.709e-06  2.529e-06  -1.862  0.0633 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
## R-sq.(adj) =  0.0132
##   Scale est. = 0.40256   n = 450
```

There is not a lot of data here so it is difficult to say a lot. GAMMs indicate a few things. For 8-OHdG, looks like moderate ketones are significantly larger than negative-small or large. No significant differences for TAC or neopterin. For cortisol, nothing is significant but both moderate and large are approaching significance. I would bet if I adjusted p-values across these models, these significances would drop out except maybe 8-OHdG. However, there are so few samples in the non-negative categories in that model that I don't feel confident saying much.

### Biomarkers and Leukocytes

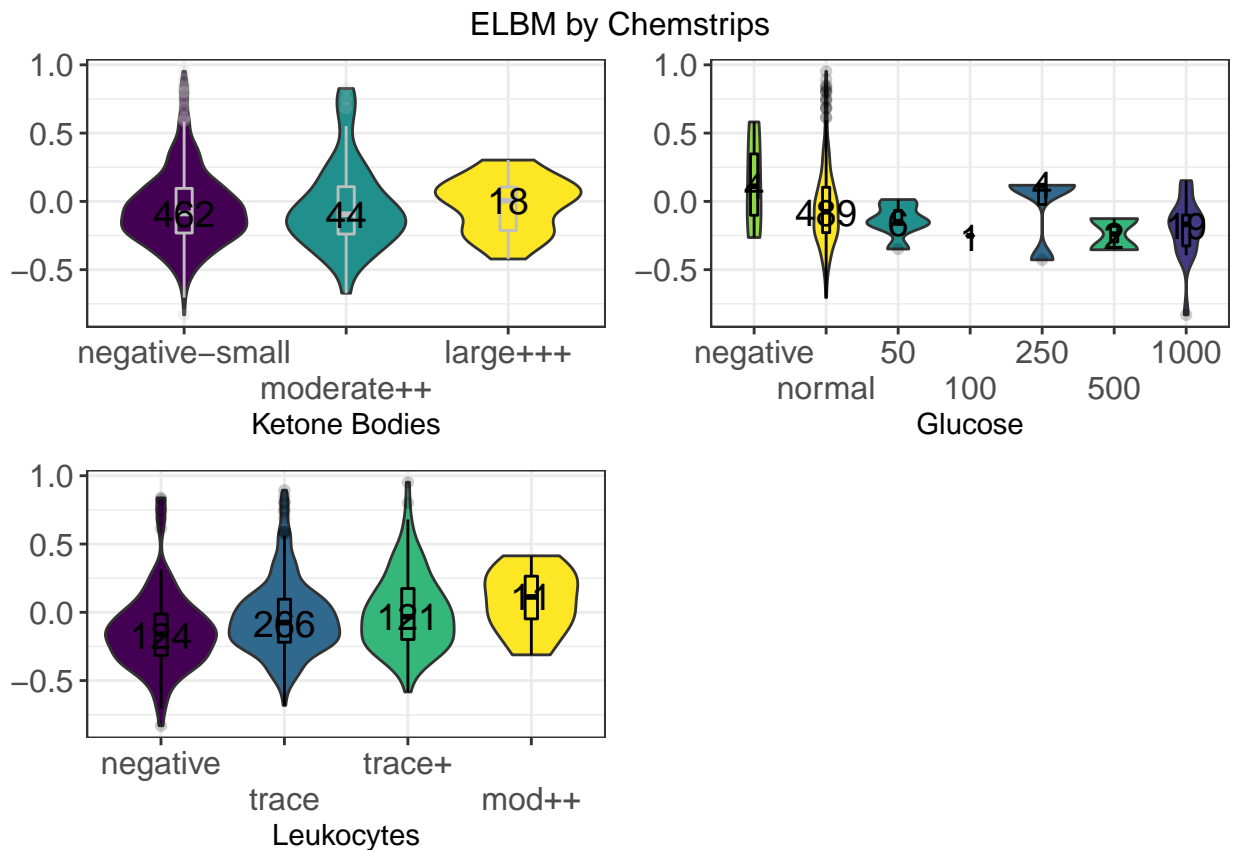


### Leukocytes

I'm not particularly seeing a relationship here. There looks like a steady positive increase in TAC might occur due to circulating leukocytes. The highest category always seems to be higher, but it has a full order of magnitude smaller a sample size so extreme caution should be taken in drawing any conclusions. The GAMM suggests this trend is indeed true. Extremely low R squared adjusted though, and estimates are quite small.

```
##
## Maximum number of PQL iterations: 20

##
## Family: Gamma
## Link function: inverse
##
## Formula:
## tac_include ~ forest_leukocytes
##
## Parametric coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.134896  0.007511  17.959  <2e-16 ***
## forest_leukocytestrace -0.003832  0.007981  -0.480   0.6314
## forest_leukocytestrace+ -0.023029  0.008979  -2.565   0.0107 *
## forest_leukocytesmod++ -0.047198  0.019161  -2.463   0.0142 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
## R-sq.(adj) =  0.0194
##   Scale est. = 0.2251    n = 402
```



Very few samples are under the moderate and large categories of ketone bodies. It would appear that some degree of increase is found in the large category. I can't really say anything about glucose. Seeing as all

categories fall within the same rough range, particularly of the negative category. If these data are accurate, I'd say there really isn't an effect. Likely the strips are simply not effective at measuring glucose. When it comes to white blood cells, there is some evidence of an increase in lean body mass. This begs the question of what the relationship between neopterin will be.

**How many samples were collected by each field worker?** This first table will include all assayed samples, broken down by type of assay. All samples are included regardless of CV, whether it was a first morning void, whether it is too dilute, or taking any other considerations.



Table 5: Table 1b: All assayed samples by which field worker collected them

biomarker	Who_collected_clean	num_collected	prop_collected
8-OHdG	Abuk	182	24.53
	Abuk, Alysse	1	0.13
	Allie	7	0.94
	Alysse	11	1.48
	Anna	2	0.27
	Beth	1	0.13
	Caco	2	0.27
	Cecilia	2	0.27
	Conor	3	0.40
	Conor, Ellie	1	0.13
	Daniel	4	0.54
	Ellie	6	0.81
	Erin	1	0.13
	Idun	135	18.19
	Idun and Tim	1	0.13
	Idun/Alysse	1	0.13
	Isman	82	11.05
	Jade	2	0.27
	Jade, Hanon?	1	0.13
	Julia	13	1.75
	Kumpo	1	0.13
	Lady	1	0.13
	Manon	2	0.27
	Misdi	1	0.13
	Paige	7	0.94
	Pawel, Kumpo	1	0.13
	Rahmadt	32	4.31
	Rahmatol	1	0.13
	Rebecca	10	1.35
	Rumaan	1	0.13
	Shaylyn	9	1.21
	Sonja	1	0.13
	Suwi	102	13.75
	Suwi, Tim	1	0.13
	Suwi/Isman	1	0.13
	Tim	4	0.54
	Tono	80	10.78
	Tono, Wendy	1	0.13
	Unknown	1	0.13
	Wendy	20	2.70
	Wilhelm	3	0.40
	Will	1	0.13
	Yann	3	0.40
	Abuk	207	20.80
	Abuk and Tim	1	0.10
	Abuk, Alysse	1	0.10
	Allie	13	1.31
	Alysse	78	7.84
	Alysse/Idun	9	0.90
	Anna	7	0.70
	Awan	1	0.10
	Beth	4	0.40
	Bonnie	1	0.10
	Brigitte	1	0.10
	Bunga	2	0.20
	Caco	17	1.71
	Cecilia	4	0.40