Effects of Dexamethasone on NCD/HFD Mouse Quadriceps

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Contents

Purpose

to examine the molecular effects of dexamethasone at varying lengths of treatment on obese and non-obese mice

Experimental Details

The relevant protocols used were:

- RNA Purification, a modified version of http://bridgeslab.sph.umich.edu/protocols/index.php?title=Preparation_of_RNA_Samples_from_Mouse_Tissues&oldid=1359
- cDNA Synthesis, used 2 ug then diluted cDNA 5X prior to qPCR: http://bridgeslab.sph.umich.edu/protocols/index.php?title=First_Strand_cDNA_Synthesis_(AB_Kit)&oldid=1242
- qPCR: http://bridgeslab.sph.umich.edu/protocols/index.php?title=QPCR&oldid=1252

There is two cohorts of mice from this, run separately on different qPCR runs.

Raw Data

The sample mapping is found in ../../data/raw/Time Course Sample Key.csv and the qPCR data can be found in ../../data/raw/Time Course Quadriceps qPCR - Combined Well Results.csv‘. This analysis can be found in /Users/davebrid/Documents/GitHub/CushingAcromegalyStudy/scripts/scripts-muscle and was most recently run on Tue Jan 7 16:23:54 2020.

Table 1: Samples where Ct range between technical replicates exceeds 1

Experiment Name	Sample Name	Target Name	Time	Diet	Ct.mean	Ct.min	Ct.max	Ct.range
2018-07-16 lg.eds	2630	GDF15	14	NCD	33.9	32.4	36.7	4.29
2018-07-16 lg.eds	2645	GDF15	3	HFD	34.9	33.1	37.3	4.13
2018-07-16 lg.eds	2618	GDF15	3	NCD	33.5	32.0	35.8	3.74
2018-07-16 lg.eds	2639	GDF15	7	NCD	34.4	33.3	36.5	3.18
2018-07-16 lg.eds	2643	GDF15	7	HFD	34.7	33.5	36.6	3.15
2018-07-16 lg.eds	2616	GDF15	3	NCD	32.2	31.3	34.4	3.11
2018-07-09 LG.eds	2645	GDF15	3	HFD	35.1	33.5	36.5	2.95
2018-07-16 lg.eds	2631	GDF15	14	NCD	33.8	32.6	35.5	2.91
2018-07-16 lg.eds	2624	GDF15	0	HFD	33.4	32.5	35.2	2.67
2018-07-16 lg.eds	2627	GDF15	0	HFD	35.1	34.3	36.9	2.67
2018-07-09 LG.eds	2626	GDF15	0	HFD	33.0	31.7	34.4	2.64
2018-07-16 lg.eds	2633	GDF15	14	HFD	35.1	33.7	36.3	2.61
2018-07-09 LG.eds	2644	GDF15	3	HFD	33.6	32.3	34.9	2.61
2018-07-09 LG.eds	2618	GDF15	3	NCD	33.5	32.6	35.2	2.60
2018-07-09 LG.eds	2639	GDF15	7	NCD	34.3	33.0	35.5	2.55
2018-07-16 lg.eds	2626	GDF15	0	HFD	34.6	33.3	35.8	2.52
2018-07-16 lg.eds	2621	GDF15	0	NCD	34.7	33.7	36.1	2.39
2018-07-16 lg.eds	2641	GDF15	7	HFD	33.6	32.3	34.7	2.33
2018-07-09 LG.eds	2629	GDF15	14	NCD	33.7	32.5	34.8	2.32
2018-07-16 lg.eds	2632	GDF15	14	HFD	34.1	33.4	35.4	2.08
2018-07-16 lg.eds	2647	GDF15	3	HFD	33.8	32.5	34.5	2.05
2018-07-09 LG.eds	2619	GDF15	3	NCD	30.7	29.6	31.7	2.03
2018-07-09 LG.eds	2633	GDF15	14	HFD	33.3	32.4	34.3	1.91
2018-07-09 LG.eds	2625	Foxo3	0	HFD	23.5	22.8	24.7	1.87
2018-07-16 lg.eds	2622	GDF15	0	NCD	32.9	32.0	33.9	1.84
2018-07-16 lg.eds	2617	GDF15	3	NCD	30.6	30.0	31.6	1.60
2018-07-09 LG.eds	2638	GDF15	7	NCD	29.9	29.2	30.7	1.47
2018-07-16 lg.eds	2644	GDF15	3	HFD	33.1	32.3	33.7	1.38
2018-07-09 LG.eds	2627	GDF15	0	HFD	36.0	35.3	36.7	1.37
2018-07-16 lg.eds	2629	GDF15	14	NCD	32.0	31.5	32.9	1.36
2018-07-09 LG.eds	2632	GDF15	14	HFD	34.4	33.7	35.1	1.34
2018-07-09 LG.eds	2625	GDF15	0	HFD	34.1	33.7	35.0	1.30
2018-07-16 lg.eds	2620	GDF15	0	NCD	34.1	33.6	34.9	1.30
2018-07-16 lg.eds	2640	GDF15	7	HFD	32.2	31.4	32.7	1.29
2018-07-09 LG.eds	2620	GDF15	0	NCD	34.2	33.6	34.9	1.28
2018-07-16 lg.eds	2625	GDF15	0	HFD	33.5	32.8	34.1	1.25
2018-07-16 lg.eds	2646	GDF15	3	HFD	33.3	32.6	33.9	1.22
2018-07-16 lg.eds	2637	GDF15	7	NCD	30.0	29.3	30.3	1.03

Table 2: Number of samples for qPCR analysis

Diet	Time	n
NCD	0	7
NCD	3	7
NCD	7	7
NCD	14	7
HFD	0	7
HFD	3	7

Diet	Time	n
HFD	7	7
HFD	14	7

We removed several wells due to technical outliers:

- J1,J6,J7,J9,J11,J12,G7,D13,D16,A15,C24,C17,J10,B21 in the first experiment.
- P6,G5,G7,G4,G1,G2,G8,G3,J10,O21,A14,B22,A20,C4,A7,B15,E7,A17,C1 in the second experiment.
- G7,P16,M13,P15 in the third experiment.
- B3,G2,F20,C1,C3,C2,C4,F3,H23,G10,E7,F14,G4,F24,G3,E15,D1,G16,F1,G12,D8 in the fourth experiment.
- A9 in the fifth experiment.

We also removed one entire amplification, Foxo1 from the third experiment as none of those samples amplified

Analysis

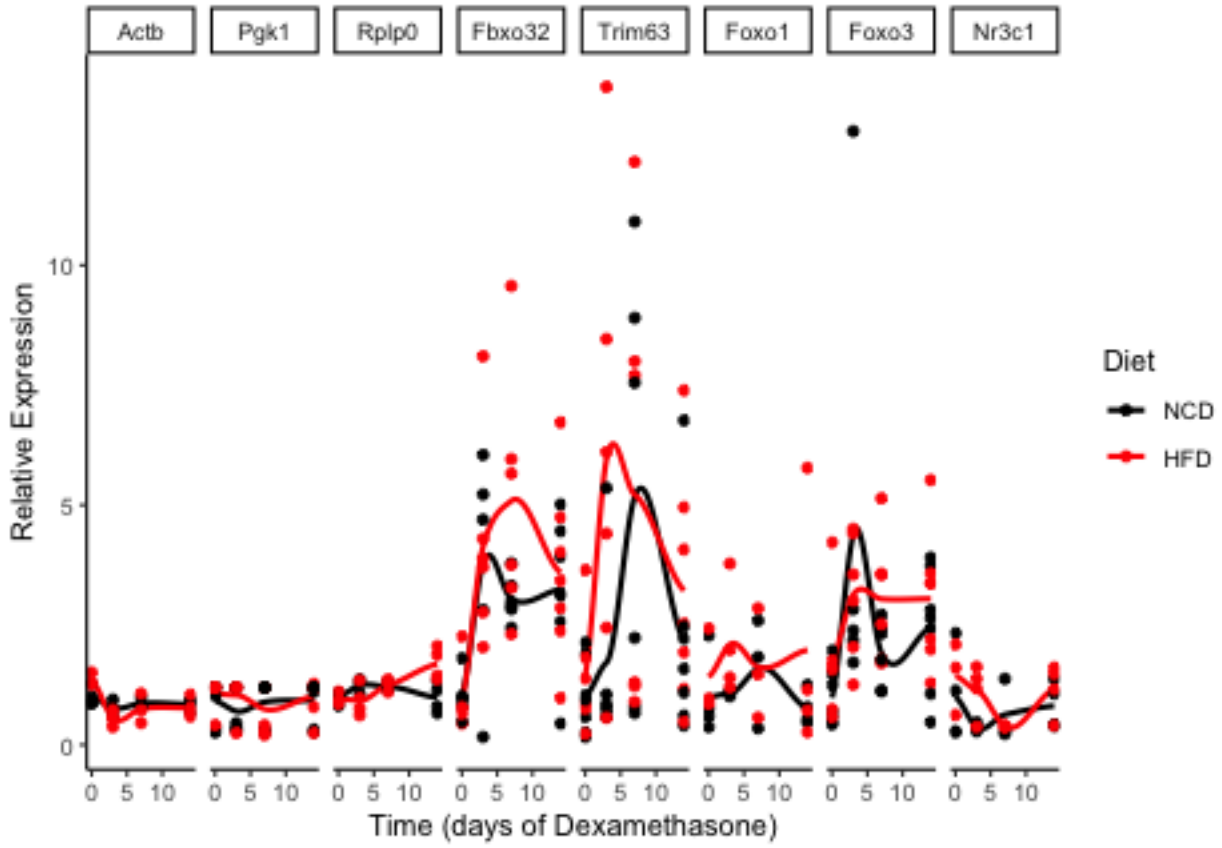
All mRNA levels were adjusted to **Pgk1**, then normalized to **NCD** at time **0**.

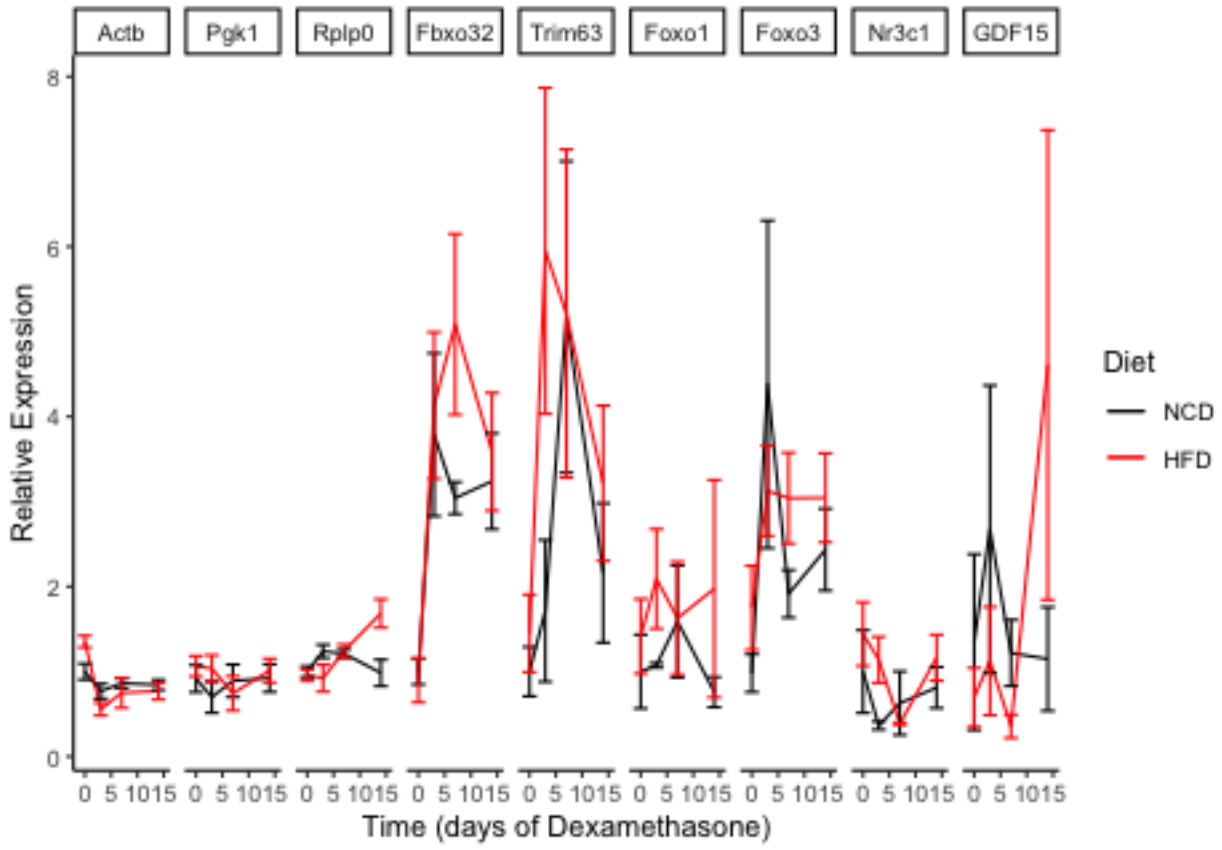
Foxo1 versus Foxo3a

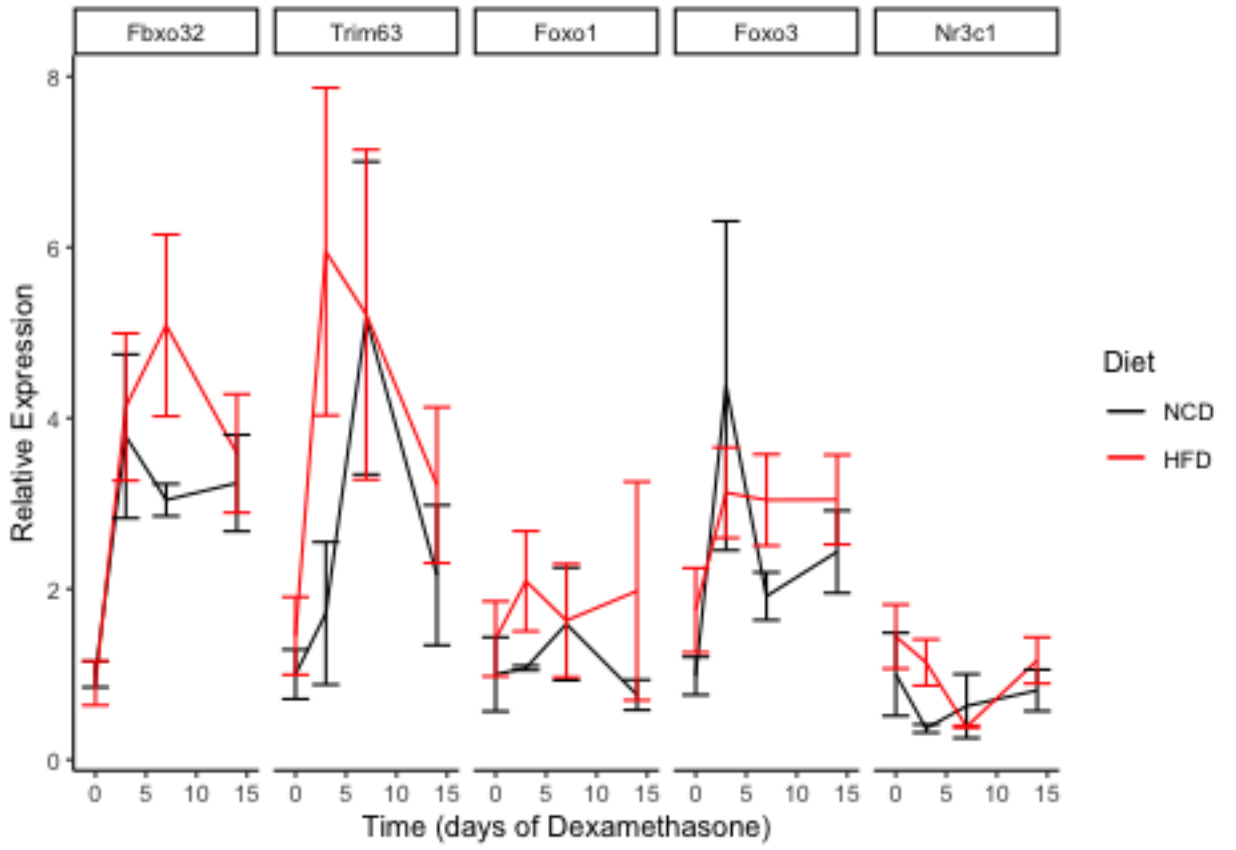
Table 3: Relative amplification of Foxo1 and Foxo3

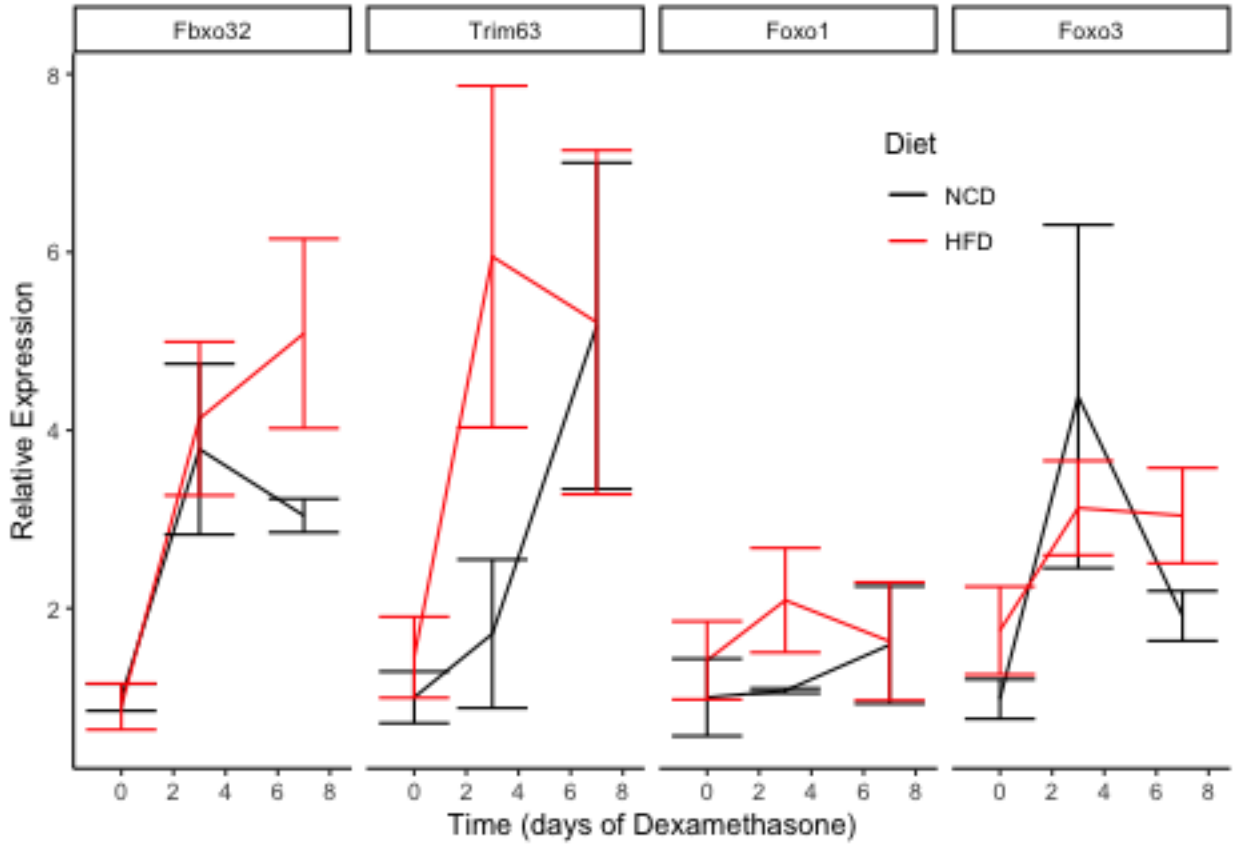
Target Name	Ct	DCt	Quant
Foxo1	24.5	0.000	1.000
Foxo3	24.7	0.262	0.834

Plots of changes in gene expression




Actb
Pgk1
Rplp0
Fbxo32
Trim63
Foxo1
Foxo3
Nr3c1
GDF15
Relative Expression
0
2
4
6
8
0 5 1015
Time (days of Dexamethasone)
Diet
NCD
HFD


Fbxo32
Trim63
Foxo1
Foxo3
Nr3c1
Relative Expression
Time (days of Dexamethasone)
Diet
NCD
HFD


Fbxo32
Trim63
Foxo1
Foxo3
Relative Expression
Time (days of Dexamethasone)
Diet
NCD
HFD

Statistics

Baseline Effects of Diet

First tested if there was an effect at baseline for expression of *Trim63* and *Fbxo32*.

Table 4: Pairwise statistics for baseline effects of diet.

Target Name	HFD.Effect	Shapiro.p	Levene.p	Wilcox.p	Welch.p	Student.p
Fbxo32	0.896	0.005	0.574	0.138	0.752	0.739
Trim63	1.450	0.306	0.515	0.731	0.452	0.431
Foxo1	1.415	0.088	0.989	0.229	0.565	0.560
Foxo3	1.784	0.075	0.440	0.181	0.226	0.187
Nr3c1	1.441	0.238	0.602	0.629	0.528	0.545
GDF15	0.518	0.011	0.538	0.886	0.587	0.574

Effects of Dexamethasone Over Time

First did pairwise tests at each time point.

Table 5: Pairwise statistics for effects of diet in dexamethasone treated animals.

Time	Target Name	HFD.Effect	Shapiro.p.HFD	Shaprio.p.NCD	Shapiro.p	Levene.p	Wilcox.p	Welch.p	Stud
0	Fbxo32	0.896	0.005	0.098	0.005	0.574	0.138	0.752	
0	Trim63	1.450	0.306	0.385	0.306	0.515	0.731	0.452	
0	Foxo1	1.415	0.119	0.088	0.088	0.989	0.229	0.565	
0	Foxo3	1.784	0.075	0.247	0.075	0.440	0.181	0.226	
0	Nr3c1	1.441	0.630	0.238	0.238	0.602	0.629	0.528	
3	Fbxo32	1.091	0.149	0.494	0.149	0.730	0.792	0.806	
3	Trim63	3.471	0.824	0.002	0.002	0.138	0.177	0.086	
3	Foxo1	1.946	0.213	0.370	0.213	0.203	0.057	0.181	
3	Foxo3	0.714	0.596	0.002	0.002	0.494	0.792	0.593	
3	Nr3c1	3.116	0.466	0.678	0.466	0.241	0.114	0.062	
7	Fbxo32	1.672	0.506	0.824	0.506	0.026	0.180	0.114	
7	Trim63	1.008	0.133	0.209	0.133	0.890	0.818	0.988	
7	Foxo1	1.025	0.772	0.650	0.650	0.986	1.000	0.968	
7	Foxo3	1.588	0.464	0.302	0.302	0.139	0.132	0.101	
7	Nr3c1	0.614	0.434	0.078	0.078	0.359	0.700	0.584	
14	Fbxo32	1.107	0.982	0.572	0.572	0.627	0.902	0.705	
14	Trim63	1.488	0.701	0.024	0.024	0.582	0.318	0.407	
14	Foxo1	2.604	0.046	0.391	0.046	0.319	0.886	0.413	
14	Foxo3	1.251	0.592	0.499	0.499	0.883	0.710	0.408	
14	Nr3c1	1.433	0.376	0.273	0.273	0.910	0.343	0.371	

Also analysed this with a linear model with diet and time as covariates, allowing for an interaction. Since time and effect were nonlinear with respect to each other, used time as a factor, allowing for each time point to be treated independently.

Table 6: Linear model for effects of time and diet on Fbxo32

term	df	sumsq	meansq	statistic	p.value
Diet	1	7.16	7.16	2.587	0.115
as.factor(Time)	3	78.71	26.24	9.478	0.000
Diet:as.factor(Time)	3	8.25	2.75	0.993	0.405
Residuals	42	116.26	2.77	NA	NA

Table 7: Linear model for effects of time and diet on Trim63

term	df	sumsq	meansq	statistic	p.value
Diet	1	26.6	26.58	2.70	0.108
as.factor(Time)	3	106.5	35.50	3.60	0.021
Diet:as.factor(Time)	3	30.8	10.26	1.04	0.384
Residuals	42	413.7	9.85	NA	NA

Table 8: Linear model for effects of time and diet on Nr3c1

term	df	sumsq	meansq	statistic	p.value
Diet	1	0.711	0.711	1.951	0.178
as.factor(Time)	3	1.766	0.589	1.616	0.217
Diet:as.factor(Time)	3	0.848	0.283	0.775	0.521
Residuals	20	7.286	0.364	NA	NA

Table 9: Linear model for effects of time and diet on Foxo1

term	df	sumsq	meansq	statistic	p.value
Diet	1	3.842	3.842	2.315	0.144
as.factor(Time)	3	0.709	0.236	0.142	0.933
Diet:as.factor(Time)	3	1.496	0.499	0.300	0.825
Residuals	20	33.199	1.660	NA	NA

Table 10: Linear model for effects of time and diet on Foxo3

term	df	sumsq	meansq	statistic	p.value
Diet	1	2.66	2.66	0.796	0.377
as.factor(Time)	3	33.17	11.05	3.307	0.029
Diet:as.factor(Time)	3	9.61	3.20	0.959	0.421
Residuals	42	140.39	3.34	NA	NA

Interpretation

None of these time courses have a significant interaction between time and diet

Session Information

```
sessionInfo()
```

```
## R version 3.5.0 (2018-04-23)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: macOS  10.15.2
##
## Matrix products: default
## BLAS: /Library/Frameworks/R.framework/Versions/3.5/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/3.5/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] broom_0.5.2      car_3.0-3        carData_3.0-2    readr_1.3.1
## [5] ggplot2_3.1.1    dplyr_0.8.3      tidyr_0.8.3.9000 knitr_1.23
##
## loaded via a namespace (and not attached):
##  [1] zip_2.0.2         Rcpp_1.0.1        cellranger_1.1.0
##  [4] pillar_1.4.2      compiler_3.5.0    plyr_1.8.4
##  [7] highr_0.8         forcats_0.4.0     tools_3.5.0
## [10] zeallot_0.1.0     digest_0.6.20     lattice_0.20-38
## [13] nlme_3.1-140      evaluate_0.14     tibble_2.1.3
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