Analysis of Glucose Clamp Data

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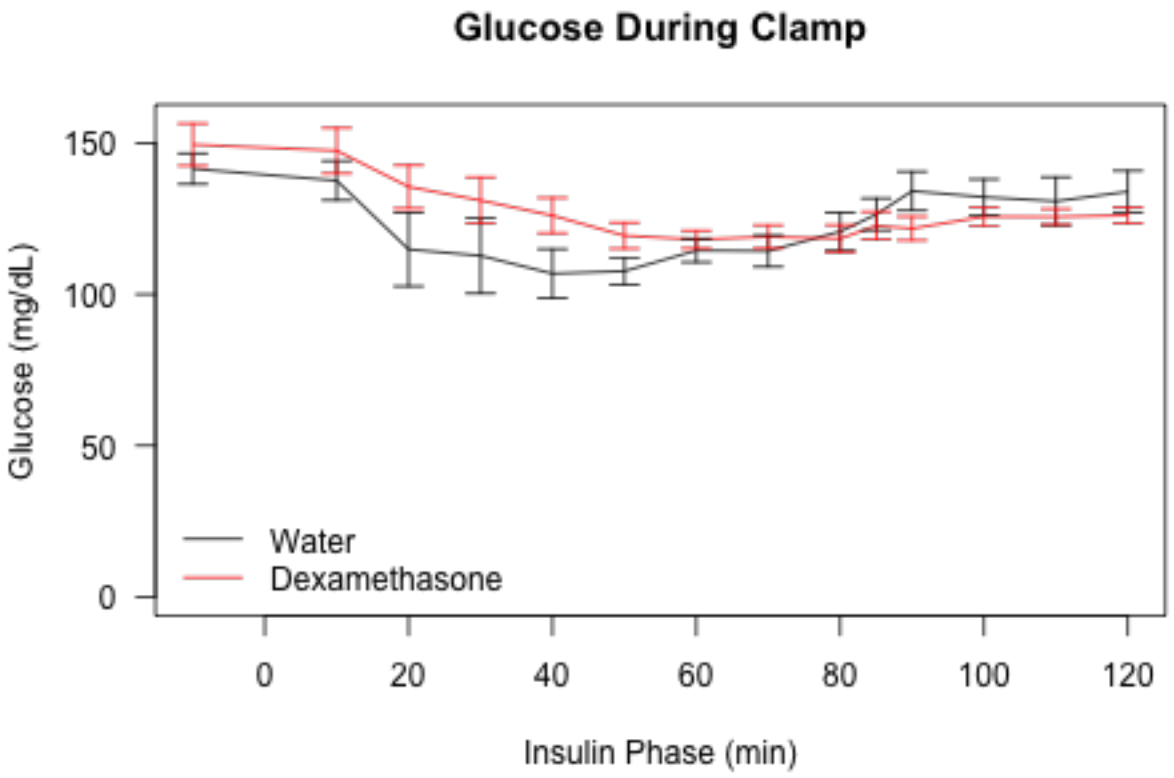
1 Data Entry

This analysis uses the averages and errors calculated first by animal, then averaged across groups. The clamp data is the average clamped values.

2 Glucose Levels During Clamp

Glucose levels were kept the same between groups throughout the experiment, as expected.



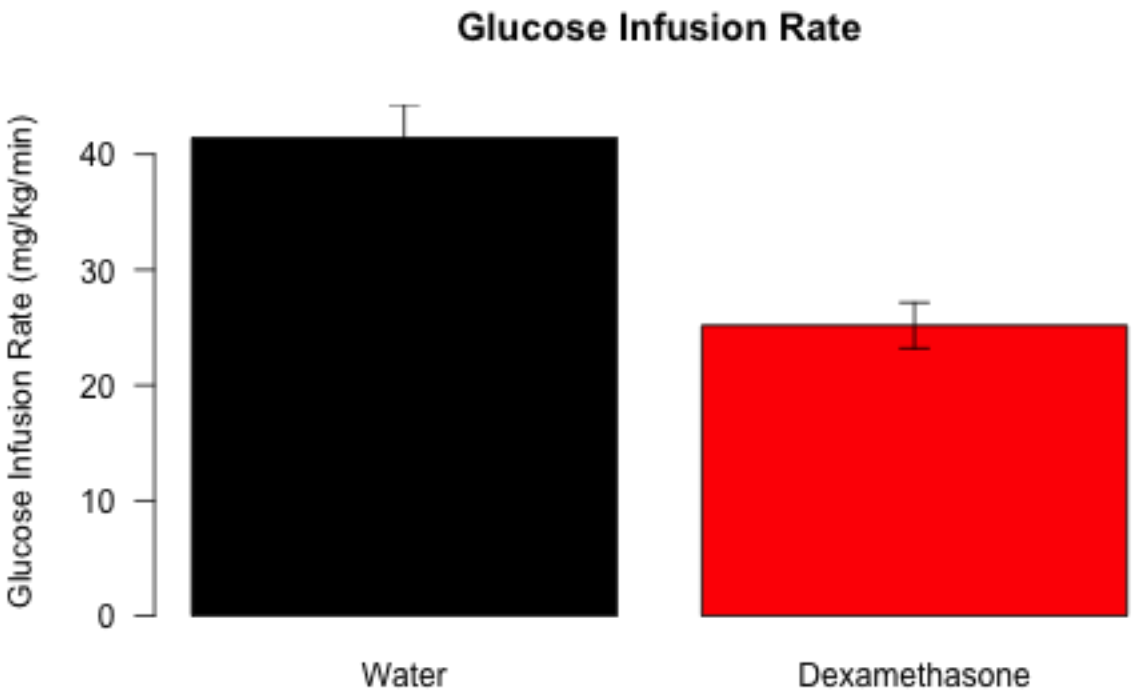
During the clamp, the goal is to keep glucose constant between 120 and 130 mg/dL throughout the experiment. There was a drop in glucose levels shortly after the insulin phase started (time 0). The following is a linear model of Glucose levels vs time, after 80 minutes showing that there was a significant effects of treatment on Glucose levels in this timeframe. The dexamethasone treated group had lower blood glucose during this phase.

Table 1: Linear model of Glucose levels at the end of the clamp phase

term	estimate	std.error	statistic	p.value
(Intercept)	119.697	5.556	21.55	0.000
Time	0.116	0.054	2.15	0.069
TreatmentDexamethasone	-7.108	1.387	-5.13	0.001

3 Glucose Infusion Rate

This only matters under insulin infusion conditions, wherein glucose levels are decreasing due to 1) suppression of endogenous production and 2) increased clearance. As such this can be thought of as a GTT such that if insulin requires more glucose infusion, that indicates more insulin sensitivity. “Glucose infusion was reduced XXX in the HFD-Dex p=XXX.”"



For the dexamethasone group, there was an average of 39.257% reduction in glucose infusion rates.

Table 2: Shapiro-Wilk Tests for Glucose Infusion Rates

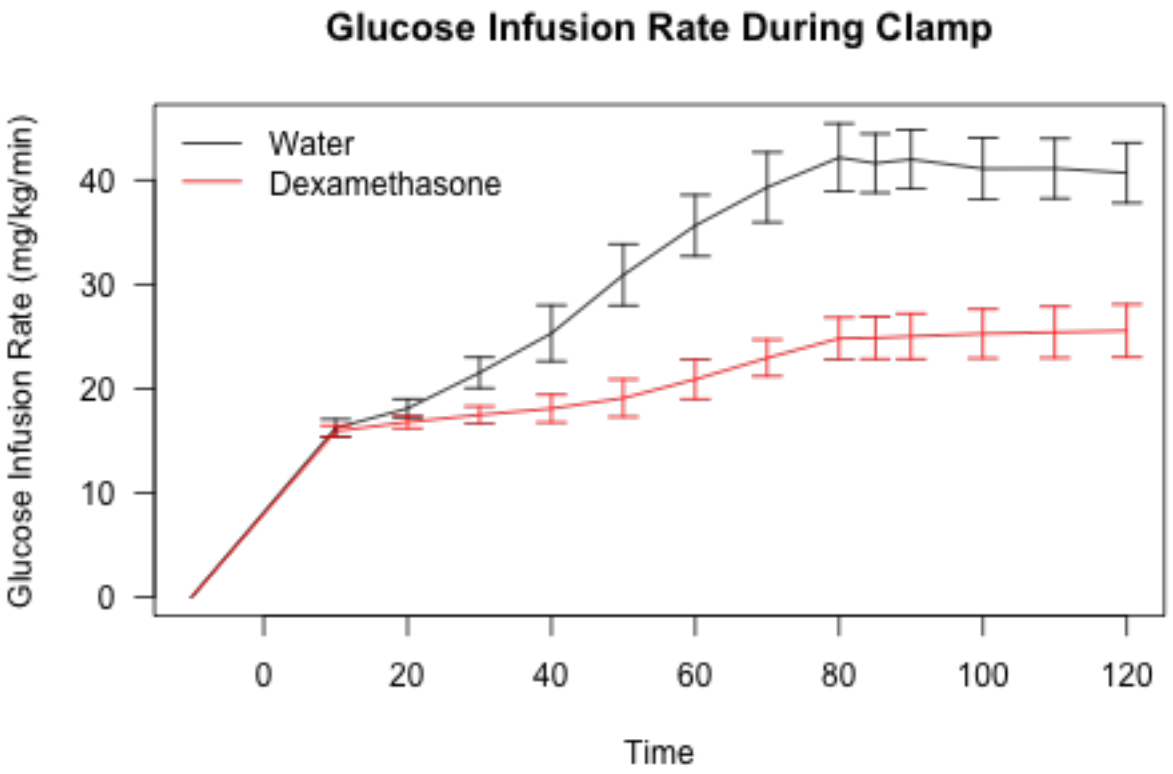
Treatment	Shapiro
Water	0.086
Dexamethasone	0.447

Table 3: Summary of t-tests for glucose infusion rates

estimate1	estimate2	statistic	p.value	parameter	conf.low	conf.high	method	alternative
41.4	25.2	4.82	0	25	9.32	23.2	Two Sample t-test	two.sided

Shapiro-Wilk tests showed that the two groups could be presumed to have equal variance, and Levene's test showed that we can assume equal variance (p=0.736) so Student's *t*-tests were used to obtain a p-value of **0**.

This is the time course for GIR over the experiment



4 Hepatic Glucose Production

This is calculated by subtracting the Glucose turnover rates from the glucose infusion rates:

$$HGP = GIR - Gtr$$

Importantly at baseline (when glucose is not changing), the glucose infusion rate is by definition zero and $Gtr = GIR$. When insulin is added, the glucose infusion rate increases to match glucose clearance (to maintain euglycemia). Now all three variables have values.'Reduced XXX in the dex but only xx in the control p=XXX'

4.1 Glucose Production in HFD

Endogenous glucose production was 36.732 percent higher in the dexamethasone-treated group in the basal condition. Under insulin stimulated conditions, endogenous glucose production was increased from nearly zero to 9.846 mg/kg/min (a -1588.054 fold increase). A Shapiro-Wilk test showed that these groups were normally distributed:

Table 4: Shapiro-Wilk Tests for Basal HGP

Treatment	Status	Shapiro
Water	Basal	0.306
Water	Insulin	0.952
Dexamethasone	Basal	0.686
Dexamethasone	Insulin	0.323

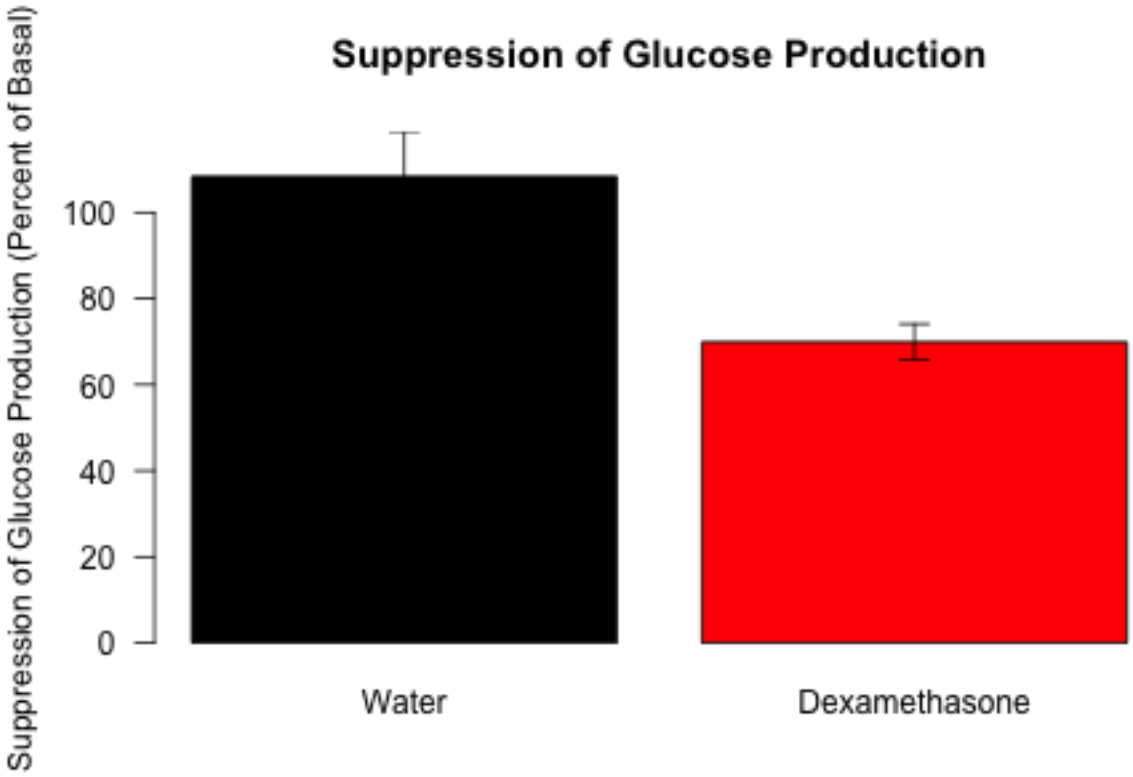
Table 5: Summary of t-tests for endogenous glucose production

	estimate1	estimate2	statistic	p.value	parameter	conf.low	conf.high	method	alternative
Basal	23.828	32.58	-2.38	0.026	23	-16.4	-1.13	Two Sample t-test	two.sided
Insulin	-0.662	9.85	-3.50	0.002	25	-16.7	-4.33	Two Sample t-test	two.sided

A Levene's test showed that both Basal (p=0.441) and Insulin phase EGP (p=0.276) can be presumed to have equal variance. Therefore a Student's *t*-test was used for both comparasons.

4.2 Suppression of Hepatic Glucose Output

Percent reductions in glucose output relative to basal were calculated for each mouse using the averaged HGP values >80 minutes



For the water group, there was an average of 108.369% reduction in glucose production, but this was only 69.905% reduction in the dexamethasone treated group.

Table 6: Shapiro-Wilk Tests for Suppression of HGP

Treatment	Shapiro
Water	0.832
Dexamethasone	0.975

Table 7: Summary of t-tests for suppression of endogenous glucose production

estimate1	estimate2	statistic	p.value	parameter	conf.low	conf.high	method	alternative
108	69.9	3.87	0.001	25	18	58.9	Two Sample t-test	two.sided

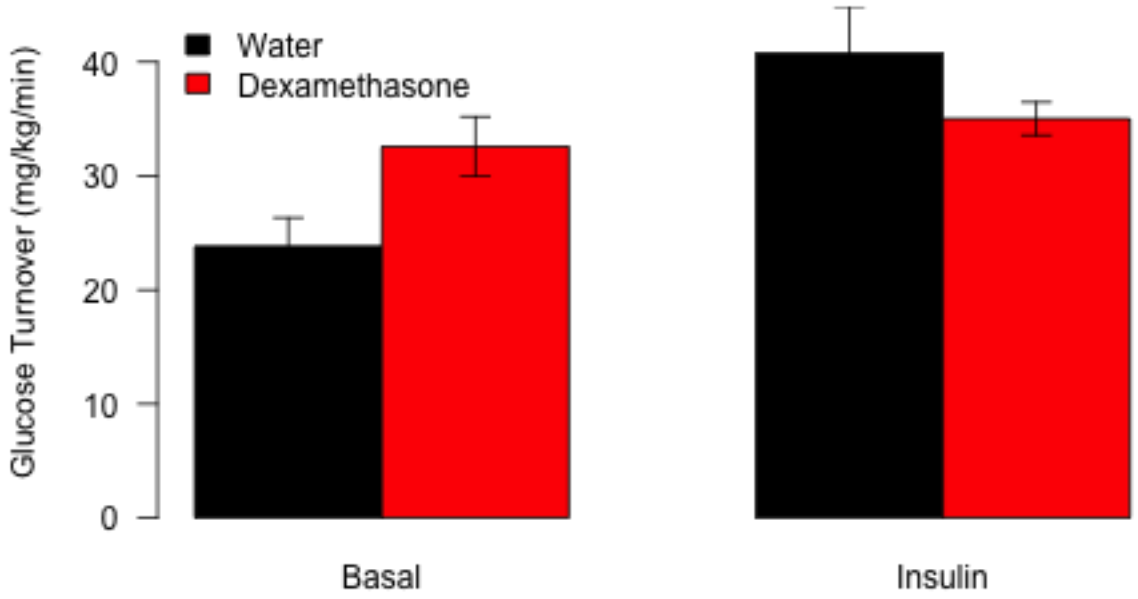
Shapiro-Wilk tests showed that the two groups could be presumed to have equal variance, and Levene's test showed that we can assume equal variance (p=0.007) so Student's *t*-tests were used to obtain a p-value of **0.001**.

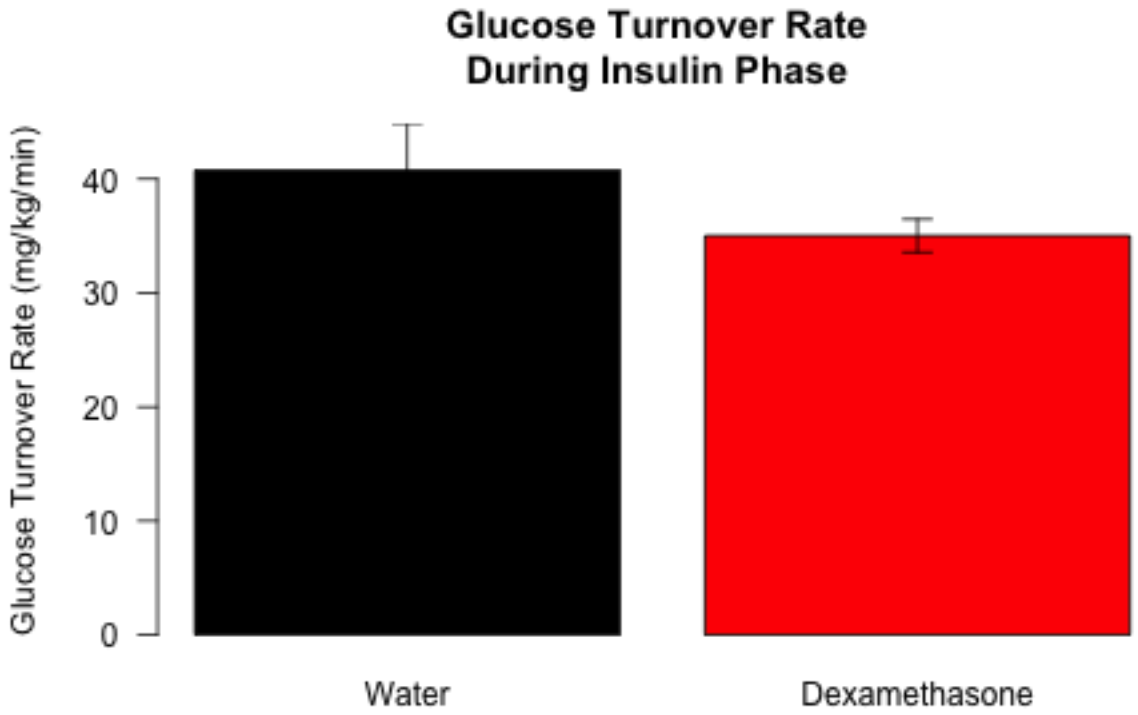
5 Glucose Turnover

This is calculated by the rate of tracer infusion divided by the specific activity of the tracer. It should increase with insulin and represents the glucose that is taken up by the body. At the basal level this is equal to the HGP since the GIR is zero.

Glucose turnover is significantly higher in HFD dex vs. controls (p=0.0262) during the basal period (not in the presence of insulin); however, this difference goes away during the clamp.

5.1 Glucose Turnover on HFD





For the dexamethasone group, there was a 14.126% reduction in glucose turnover during the insulin phase.

Table 8: Shapiro-Wilk Tests for glucose turnover during the insulin phase

Treatment	Shapiro
Water	0.536
Dexamethasone	0.501

Table 9: Summary of t-tests for glucose turnover during the insulin phase

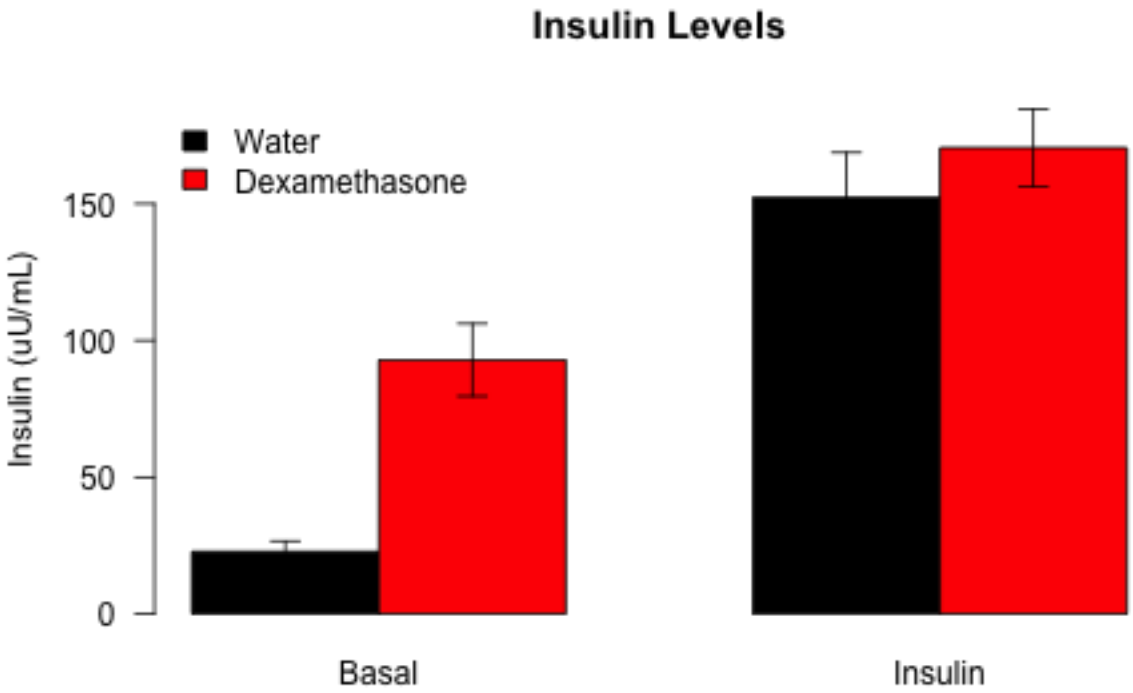
estimate1	estimate2	statistic	p.value	parameter	conf.low	conf.high	method	alternative
40.8	35	1.52	0.141	25	-2.05	13.6	Two Sample t-test	two.sided

Shapiro-Wilk tests showed that the two groups could be presumed to have equal variance, and Levene's test showed that we can assume equal variance (p=0.027) so Student's *t*-tests were used to obtain a p-value of **0.141**.

6 Insulin and Glucose Levels During the Clamp

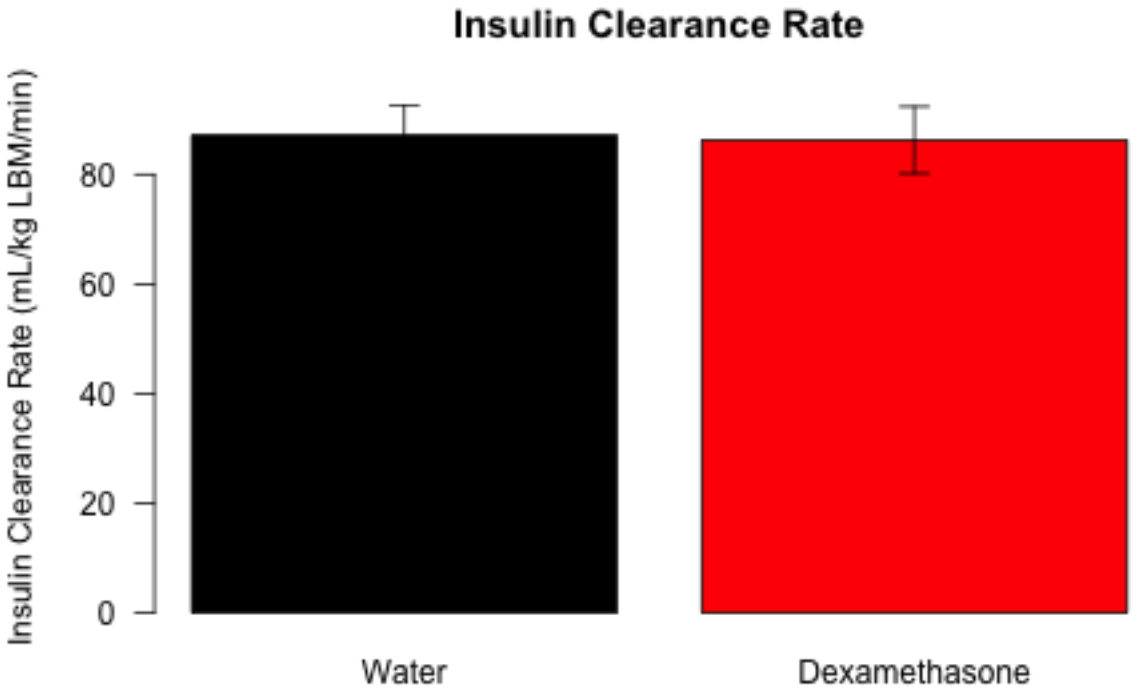
6.1 Insulin levels

Insulin levels were significantly higher in the basal period in the dex-treated mice (p=0.0001) there was no difference between the groups during the clamp.



6.2 Insulin Clearance Rates

There was no difference in insulin clearance between the groups



For the dexamethasone group, there was only a 1.039% reduction in glucose infusion rates.

Table 10: Shapiro-Wilk Tests for insulin clearance rates

Treatment	Shapiro
Water	0.226
Dexamethasone	0.990

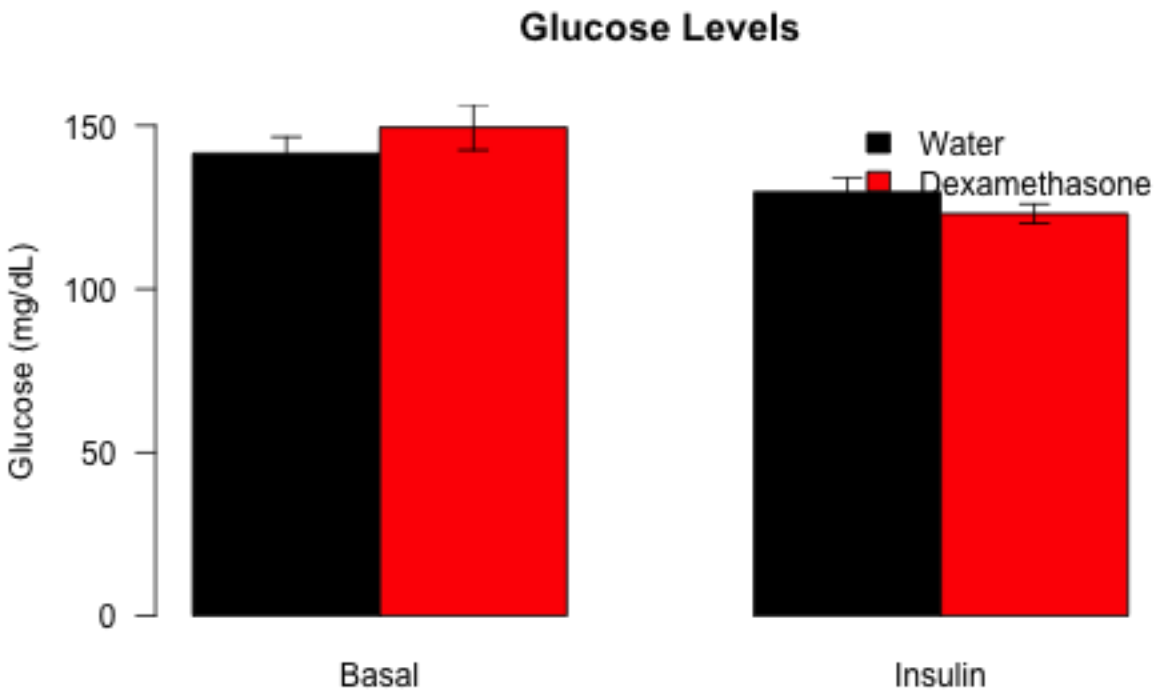
Table 11: Summary of t-tests for insulin clearance rates

estimate1	estimate2	statistic	p.value	parameter	conf.low	conf.high	method	alternative
87.2	86.3	0.108	0.915	23	-16.5	18.3	Two Sample t-test	two.sided

Shapiro-Wilk tests showed that the two groups could be presumed to have equal variance, and Levene's test showed that we can assume equal variance (p=0.268) so Student's *t*-tests were used to obtain a p-value of **0.915**.

6.3 Glucose Levels

Glucose was kept similar between the groups throughout the experiment



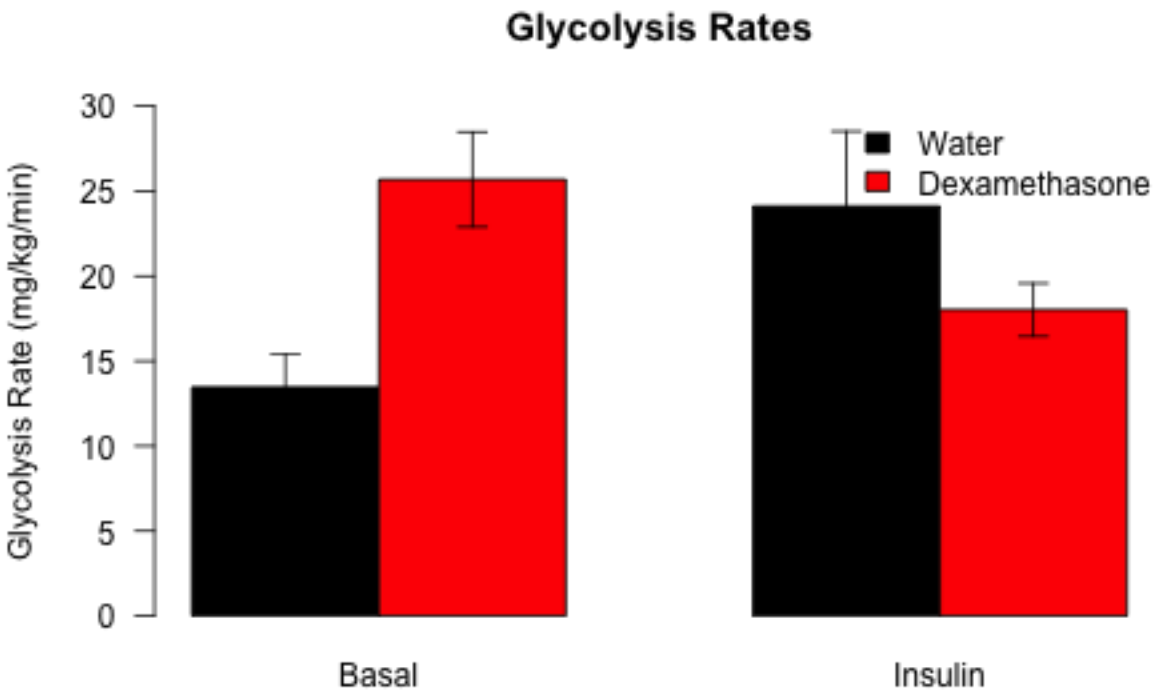
7 2-Deoxyglucose Uptake in Tissues

7.1 Inguinal Adipose Tissue

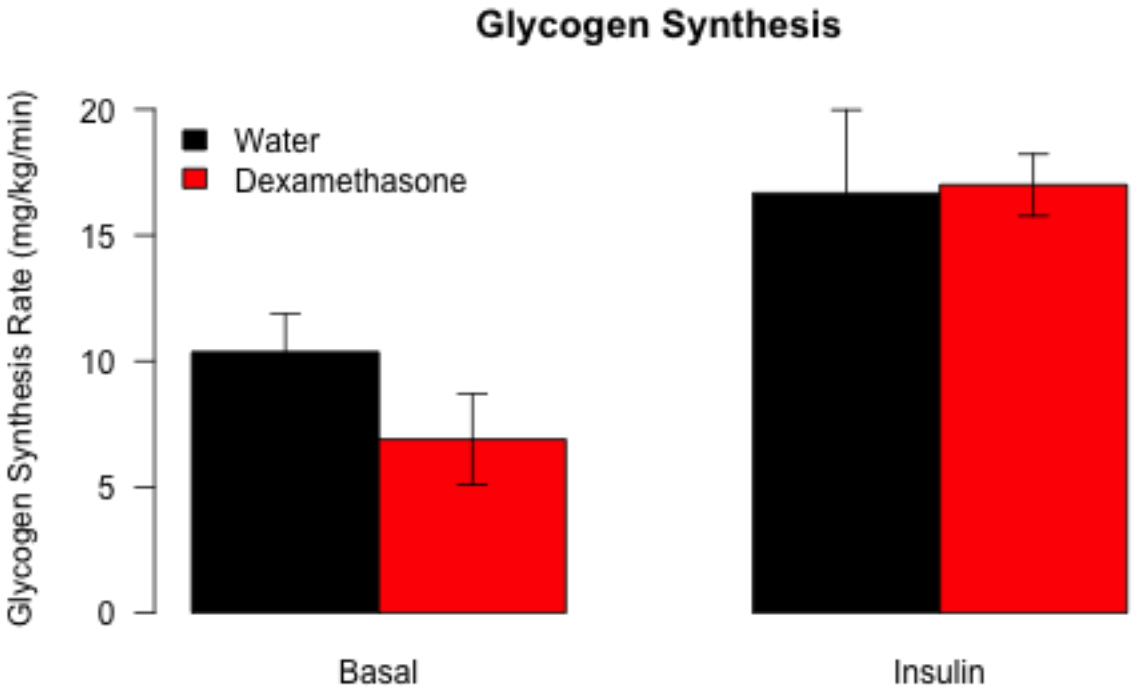
Need to make new tables for glucose uptake data

8 Glucose Disposal Metabolism

8.1 Glycolysis



8.2 Glycogen Synthesis Rate



9 Session Information

```
## R version 3.3.0 (2016-05-03)
## Platform: x86_64-apple-darwin13.4.0 (64-bit)
## Running under: OS X 10.12.6 (unknown)
##
## 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] car_2.1-4    broom_0.4.2  readr_1.1.0  dplyr_0.5.0  tidyr_0.6.1
## [6] knitr_1.15.1
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_0.12.10       nloptr_1.0.4       plyr_1.8.4
##  [4] highr_0.6          tools_3.3.0        digest_0.6.12
##  [7] lme4_1.1-12        evaluate_0.10      tibble_1.3.0
## [10] nlme_3.1-131       lattice_0.20-35    mgcv_1.8-17
## [13] Matrix_1.2-8       psych_1.7.3.21     DBI_0.6-1
```

```
## [16] yaml_2.1.14        parallel_3.3.0     SparseM_1.76
## [19] stringr_1.2.0      MatrixModels_0.4-1 hms_0.3
## [22] rprojroot_1.2      grid_3.3.0         nnet_7.3-12
## [25] R6_2.2.0           foreign_0.8-67     rmarkdown_1.6
## [28] minqa_1.2.4        reshape2_1.4.2     magrittr_1.5
## [31] backports_1.0.5    htmltools_0.3.5    MASS_7.3-45
## [34] splines_3.3.0      assertthat_0.1     pbkrtest_0.4-7
## [37] mnormt_1.5-5       quantreg_5.29      stringi_1.1.3
## [40] lazyeval_0.2.0
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