Effects of Increased Mitochondrial DNA Mutations in Diabetic Mice

Raymond Fox

Smithies-Maeda Laboratory

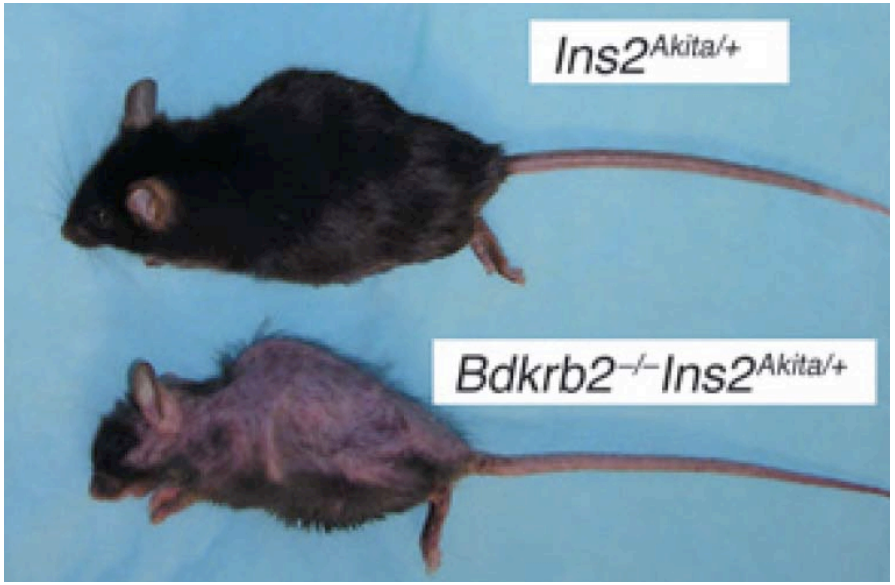
Why Mitochondrial DNA Mutations?

- Diabetic nephropathy is markedly enhanced in mice lacking the bradykinin receptor

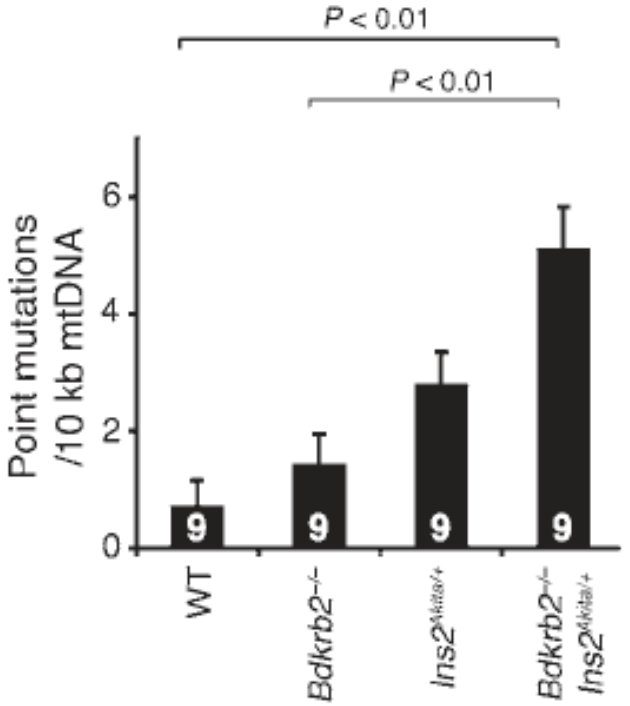
  (Kakoki, et al PNAS 2004 vol 101 no 36)

- Senescence-associated phenotypes in Akita diabetic mice are enhanced by absence of bradykinin B2 receptors

  (Kakoki et al JCI 2006 vol 116 no 5)



12 Month Old



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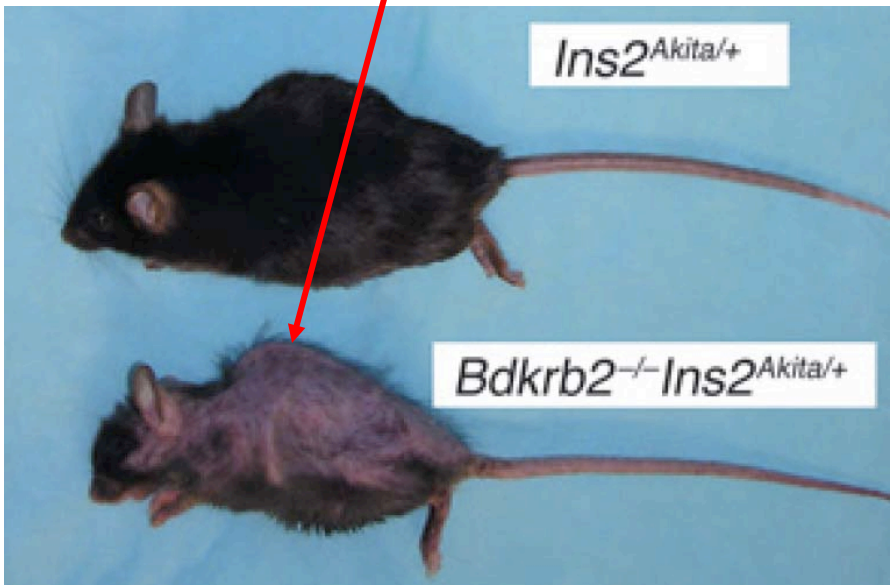

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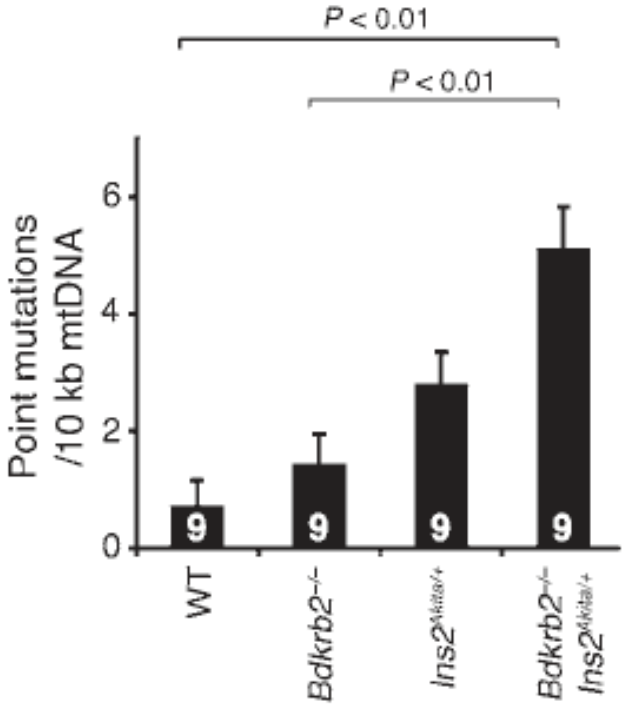

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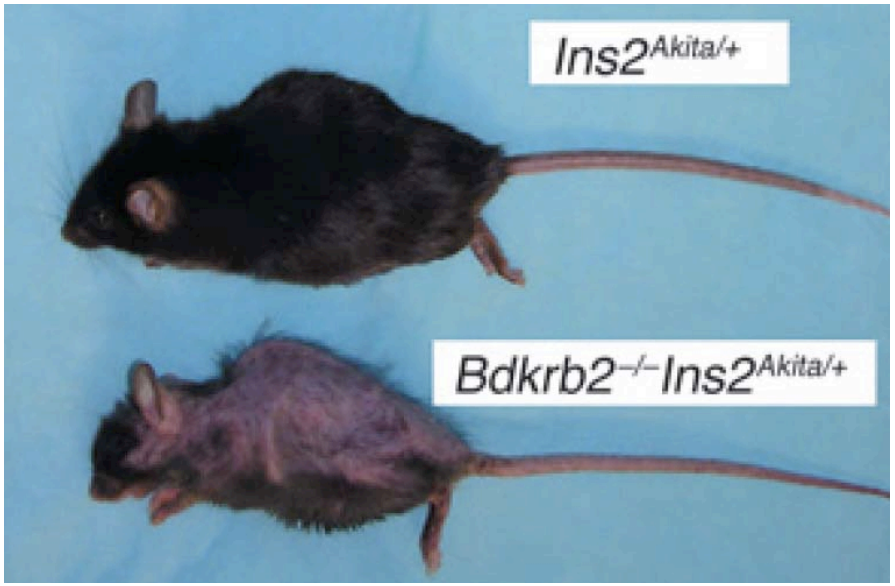

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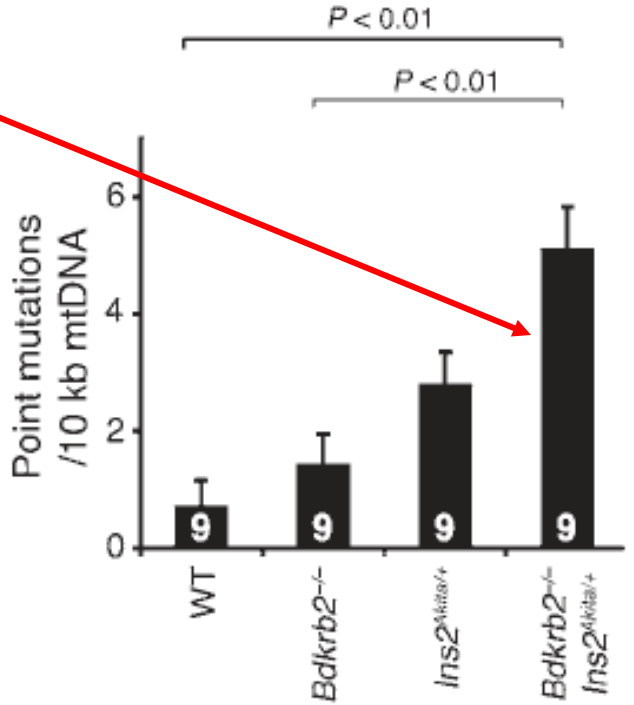


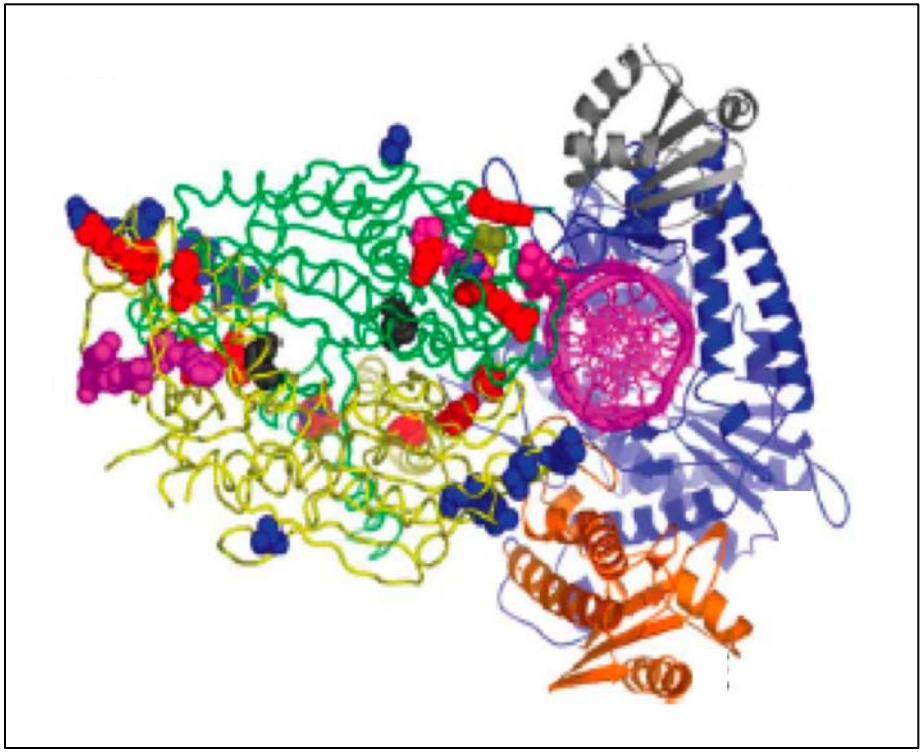
Polymerase gamma (*Polg*)

- *Polg* is essential for replication and proof reading of mitochondrial (mt) DNA.
- *Polg*$^{-/-}$ is an embryonic lethal mutation.

  (Hance, et al Human Molecular Genetics 2005 v14 1775-1783)

- A mutation in the exonuclease domain (II) at D257A disrupts the proof reading ability.

  (Kujoth, et al Science 2005 vol 309 481-484 and Trifunovic, et al Nature 2004 v429 417-423)



Fan, et al. J. Mol. Biol. (2006) 358, 1229-1243

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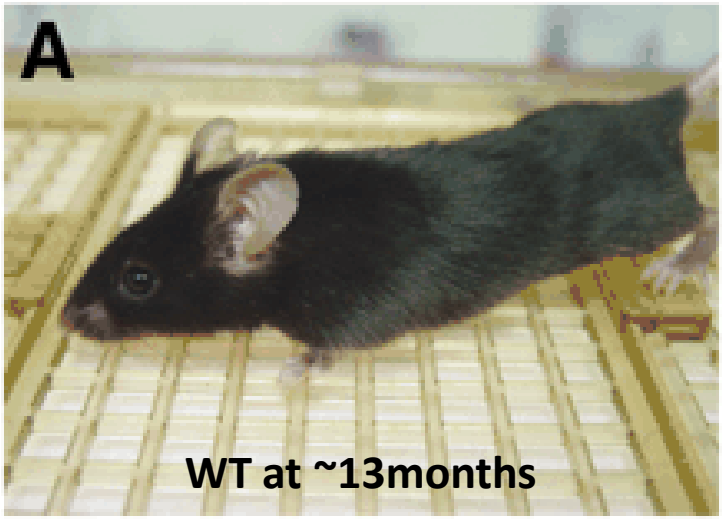
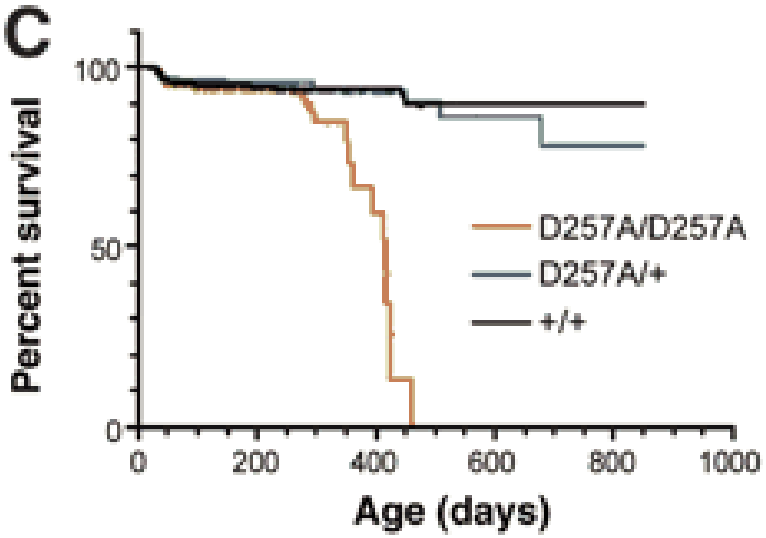
Premature Aging of the Mutant *Polg*^{D257A} Mouse



Unique Characteristics of the homozygous *Polg* mutant mouse

- **Graying of hair, loss of hair, and kyphosis (hunch back)**
- **No elevation in oxidative stress**
- **A general increase in apoptosis**
- **Reduced lifespan (~13 months)**



Kujoth, et al Science 2005 vol 309 481-484

Our Question:

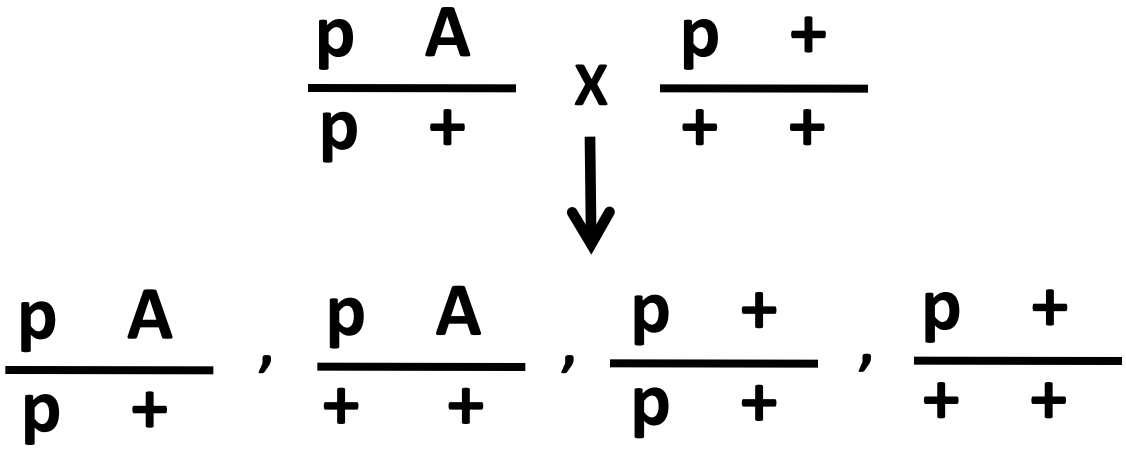
Will Akita diabetic mice with the *Polg* editing mutation develop worse complications?

Mouse Breeding Complications

- *Polg* and *Ins2* located on same chromosome (7)
- Therefore combining the mutations required extensive breeding
- Subsequent crossovers further complicated the breeding

Ray Fox's Breeding Scheme

p = *Polg*^{D257A}
A = *Ins2*^{C96Y} (Akita)
+ = Wild type



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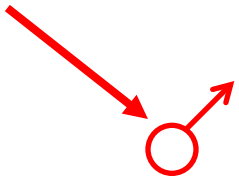

$$\frac{p\ \ A}{p\ \ +} \times \frac{p\ \ +}{+\ \ +}$$

$$\frac{p\ \ A}{p\ \ +}\ ,\ \frac{p\ \ A}{+\ \ +}\ ,\ \frac{p\ \ +}{p\ \ +}\ ,\ \frac{p\ \ +}{+\ \ +}$$

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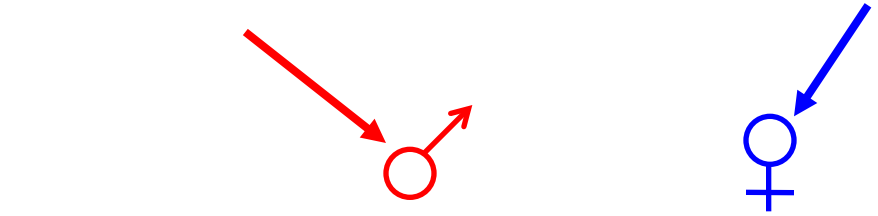

$$\frac{p\ A}{p\ +} \times \frac{p\ +}{+\ +}$$

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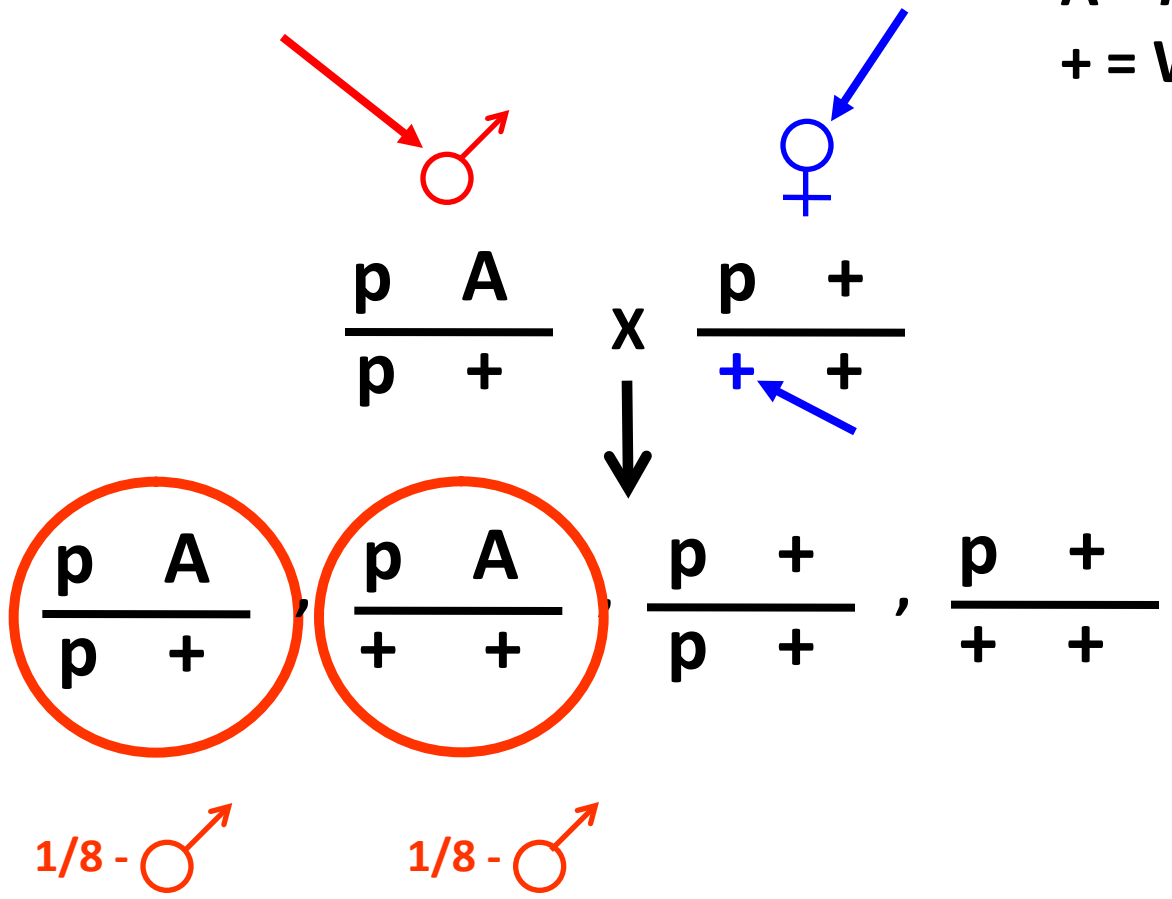
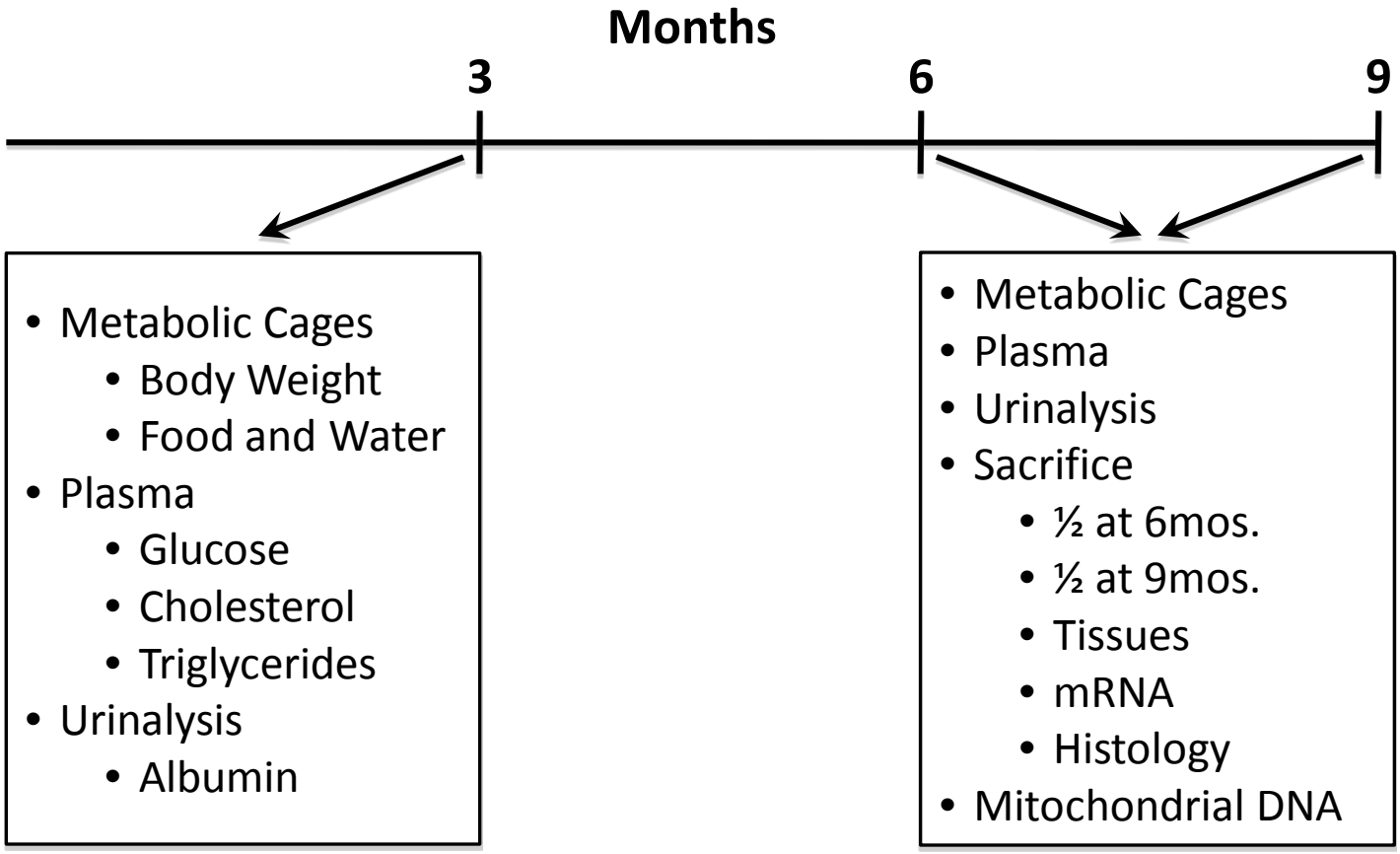


Experimental Design

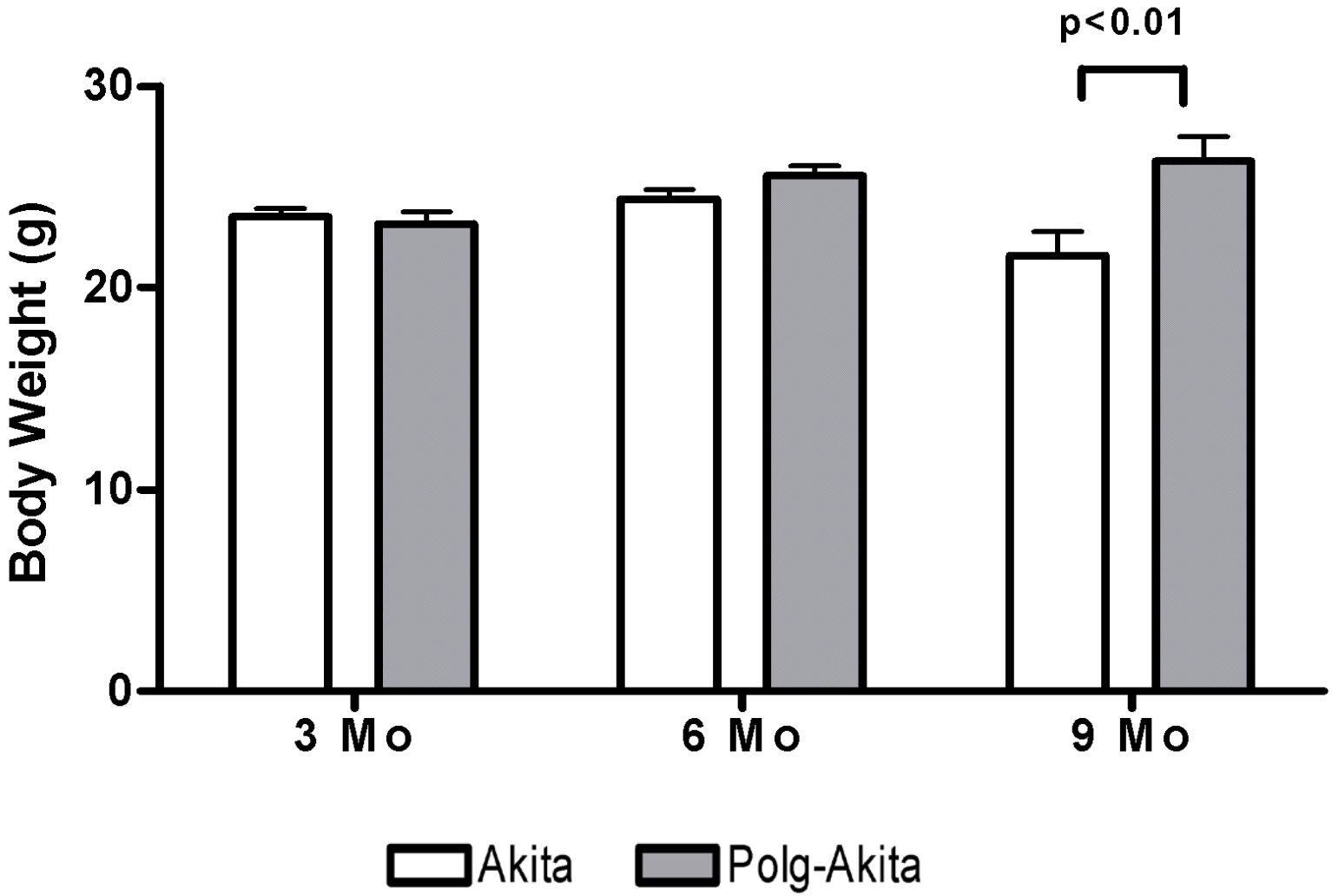
Polg-Akita vs. Akita



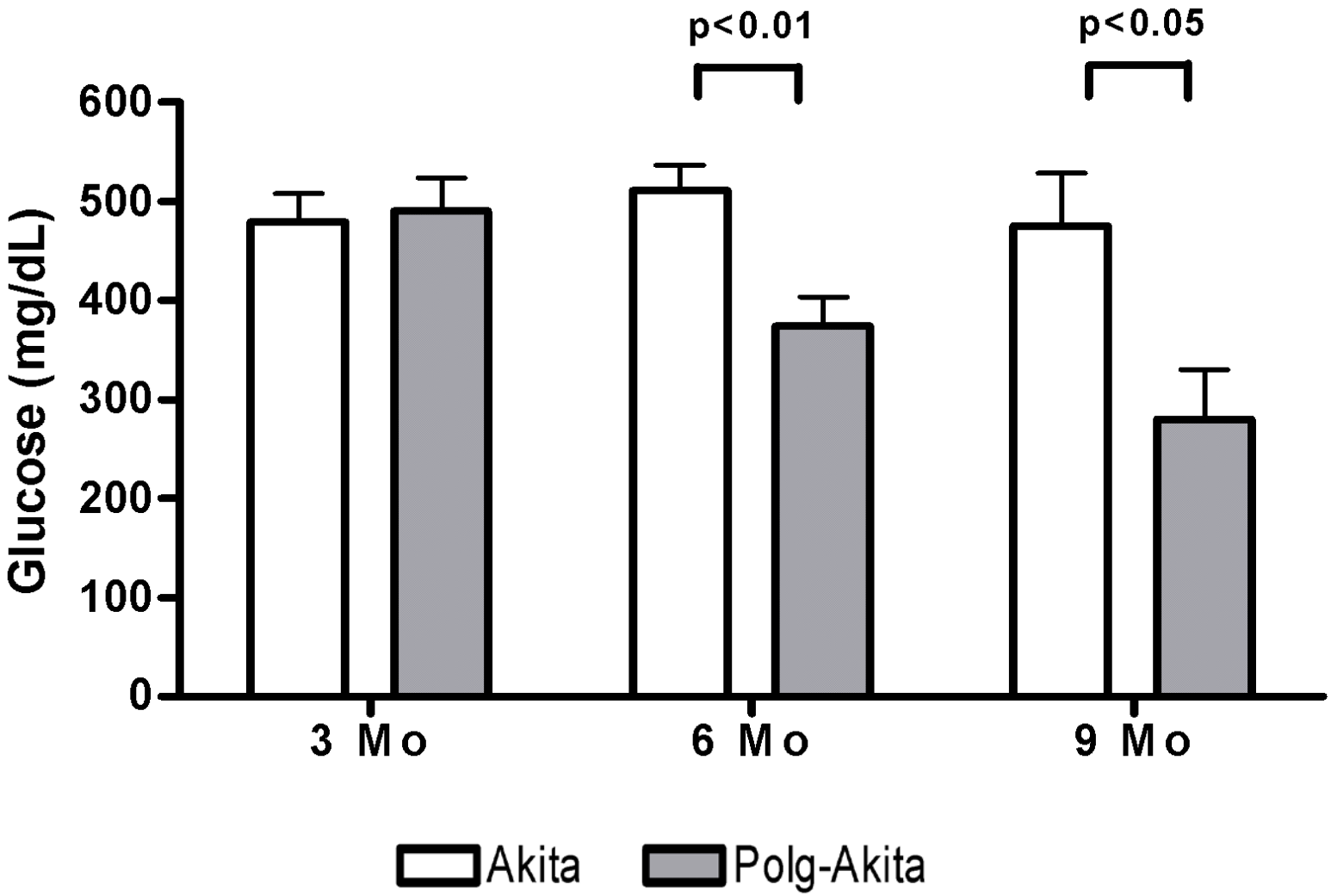
Result

Global mtDNA mutations *decrease* the diabetic phenotype

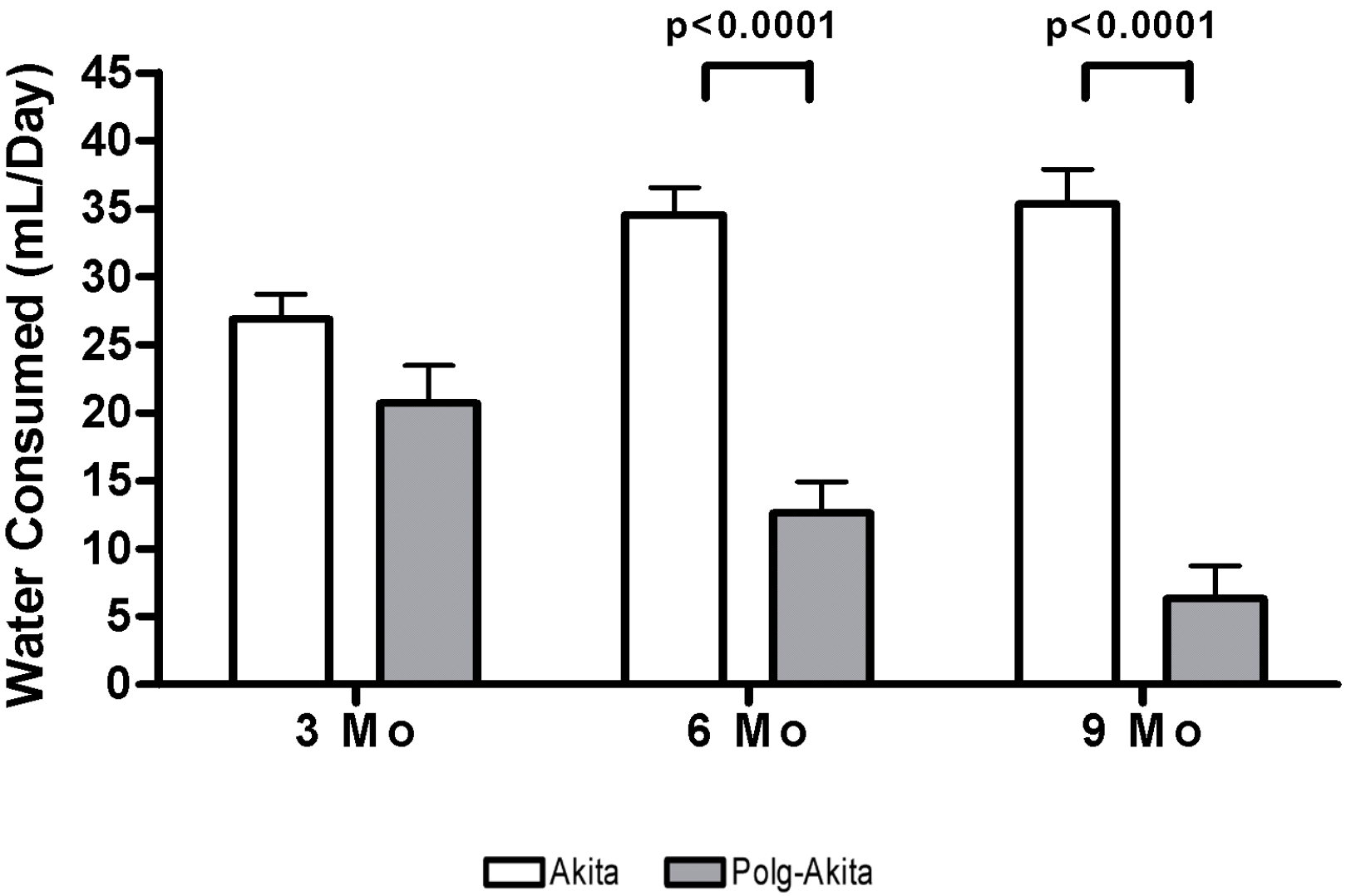
Body Weight



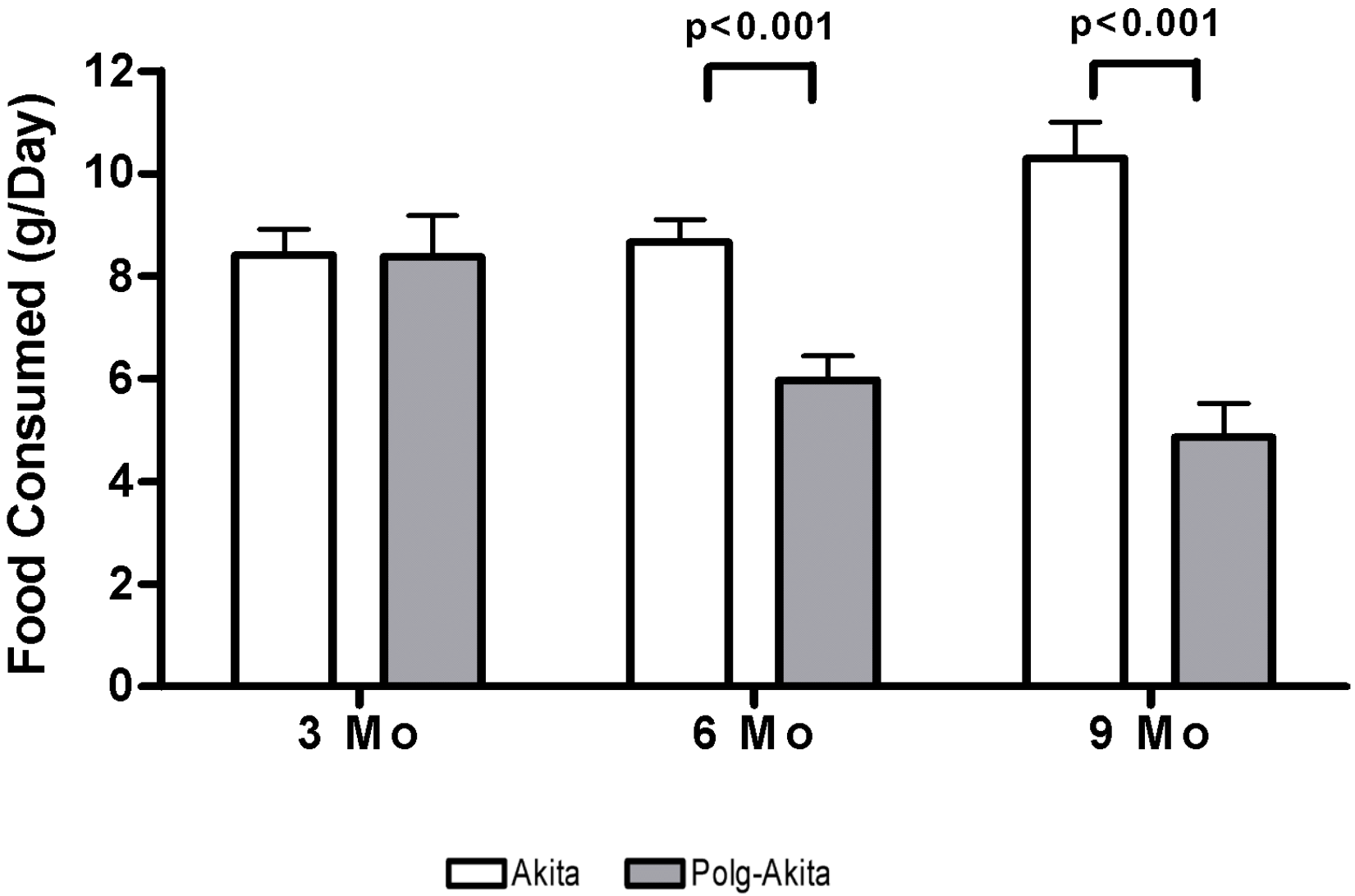
Plasma Glucose

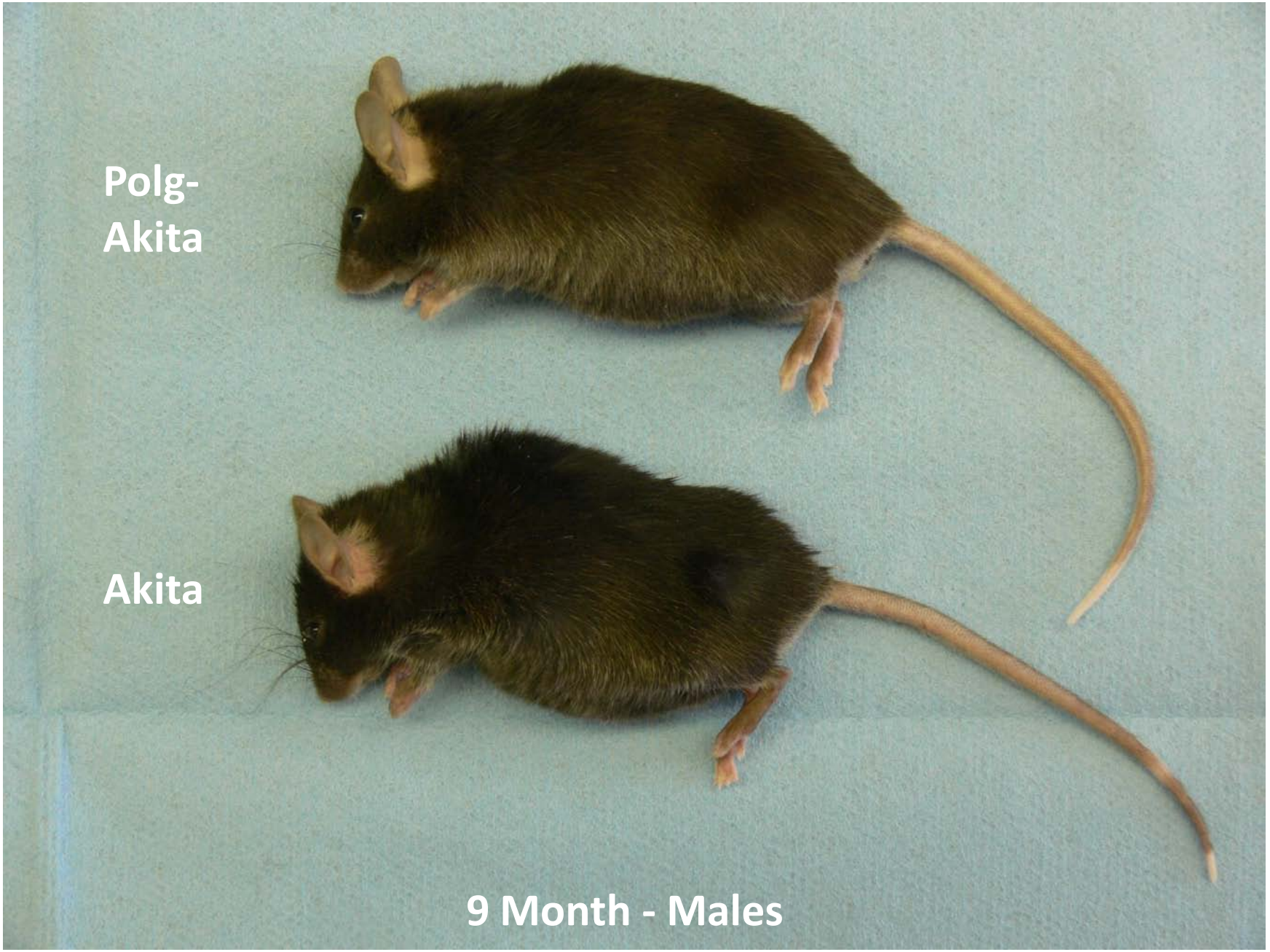


Water Intake

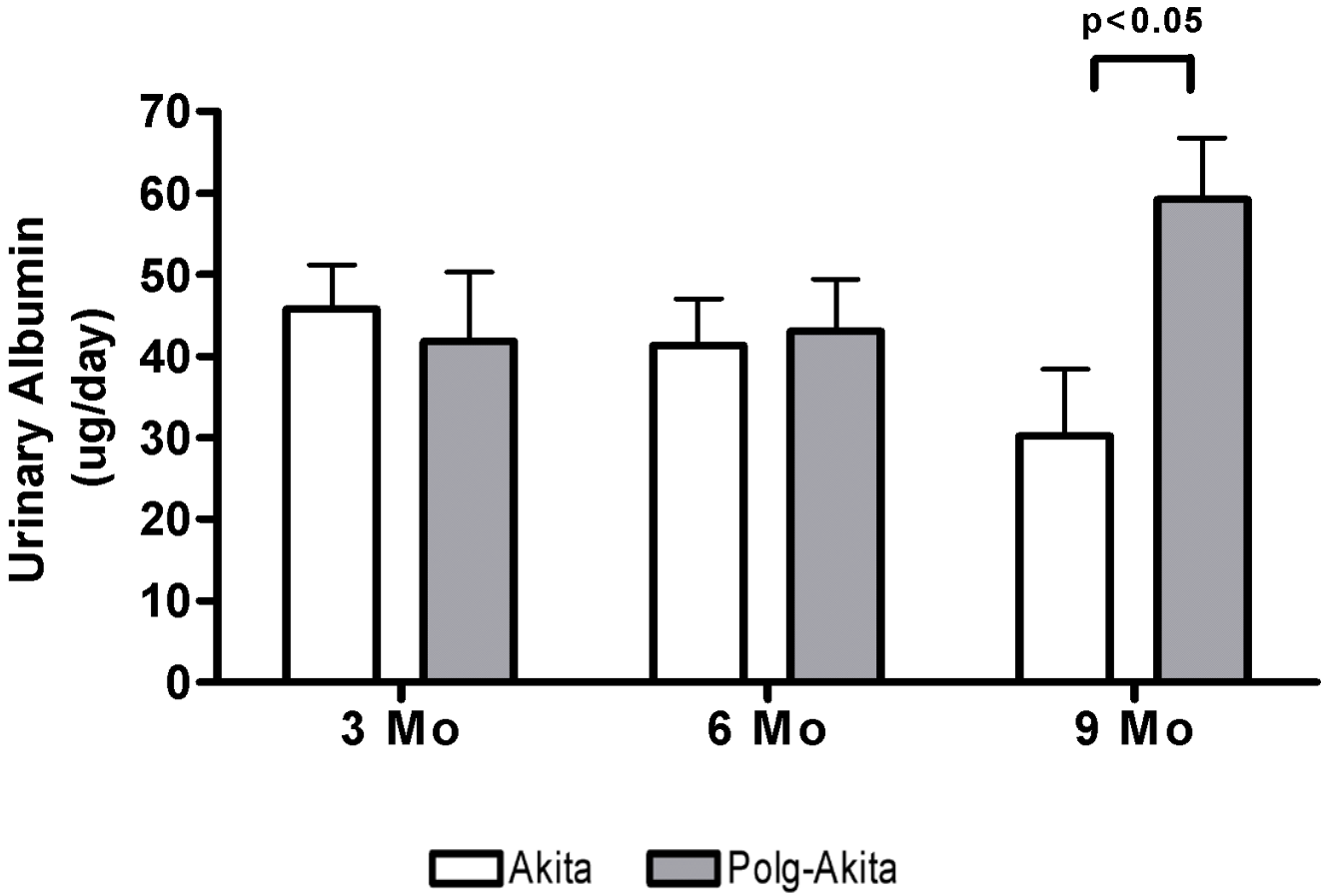


Food Intake

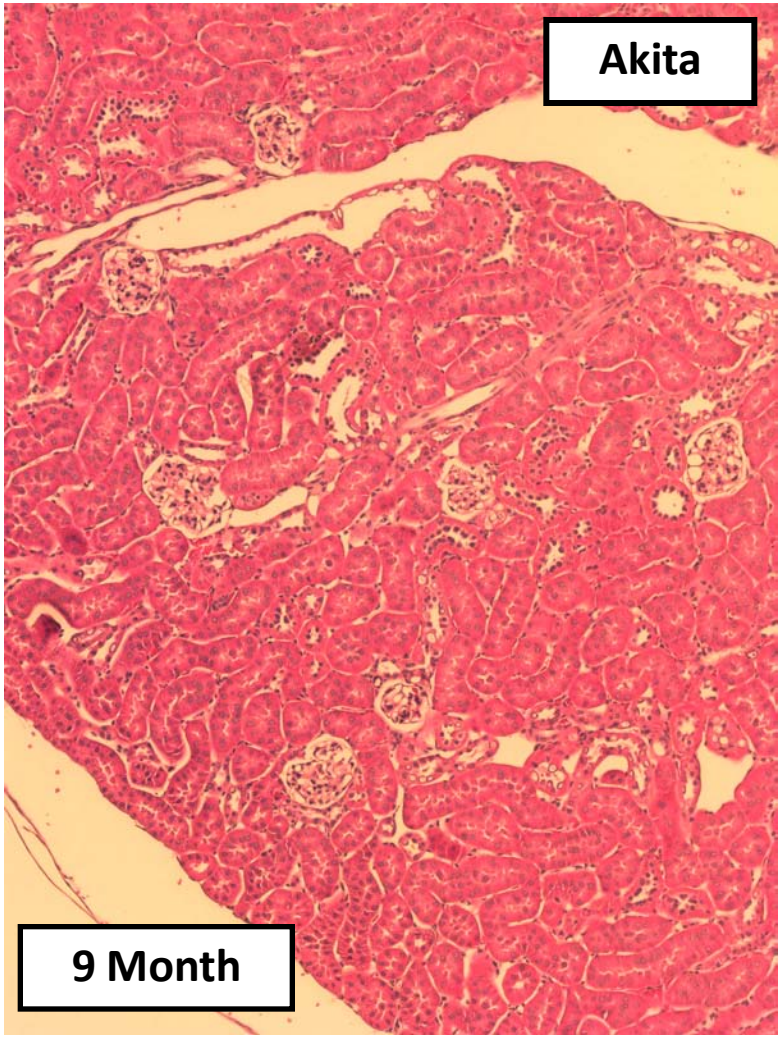

p<0.001
p<0.001
Food Consumed (g/Day)
12
10
8
6
4
2
0
3 Mo
6 Mo
9 Mo
Akita
Polg-Akita

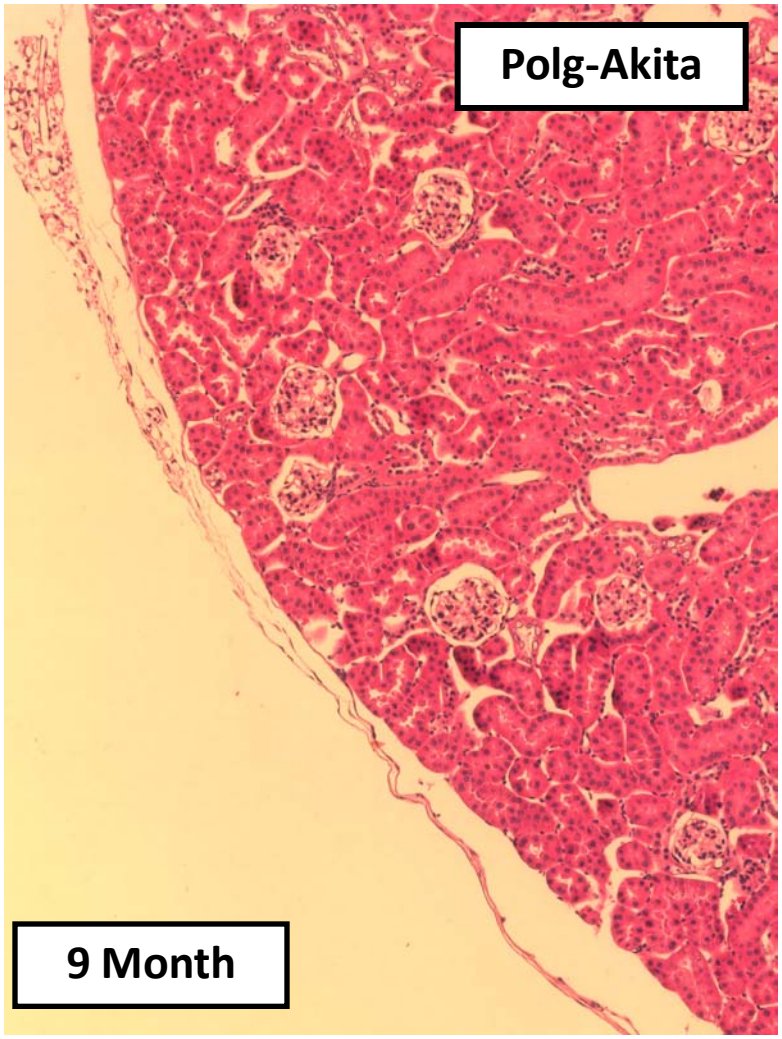

Polg-
Akita
Akita
9 Month - Males

Urinary Albumin



Kidney Histology





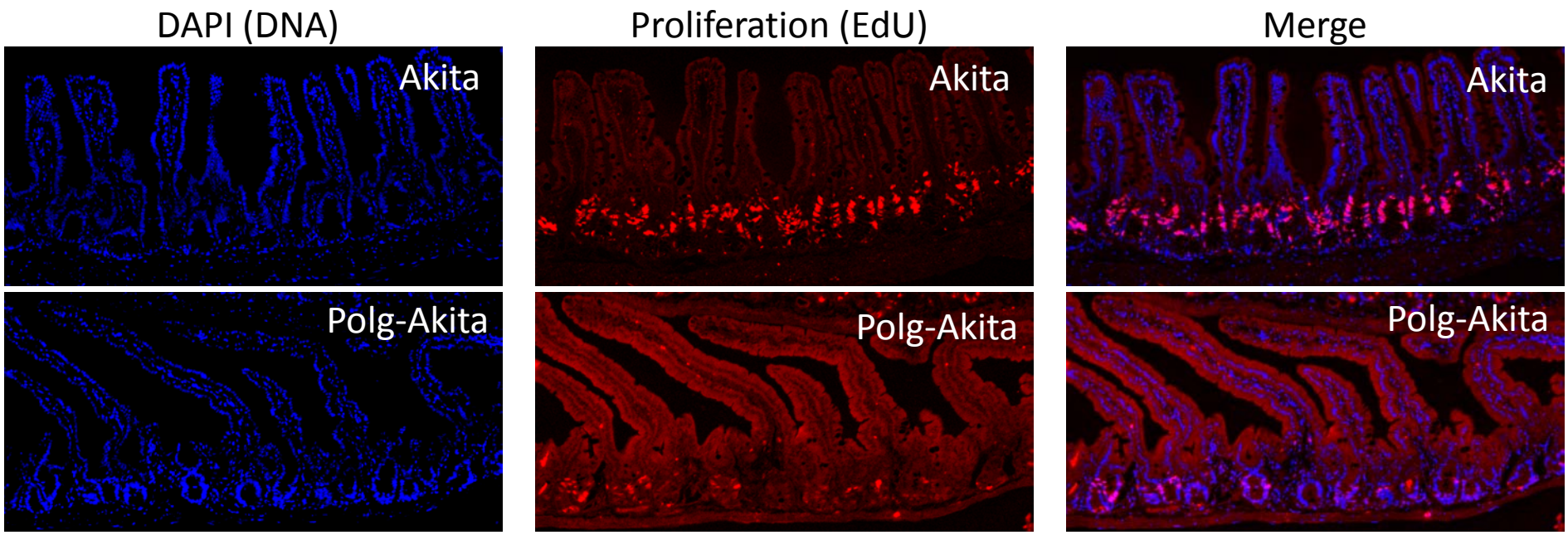
Why are the Polg-Akita mice displaying a reduced diabetic phenotype?

1. Small intestine absorption defect due to increased apoptosis and/or decreased proliferation?

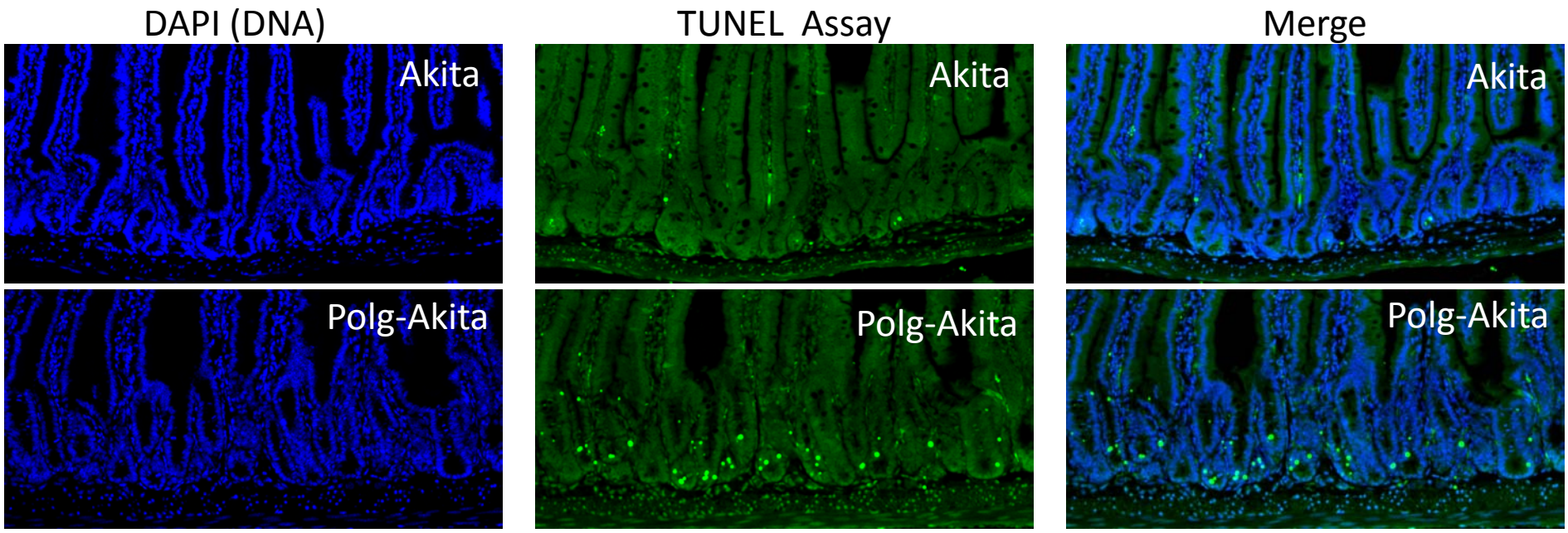
2. Testis malfunction leading to changes in brain signaling for appetite?

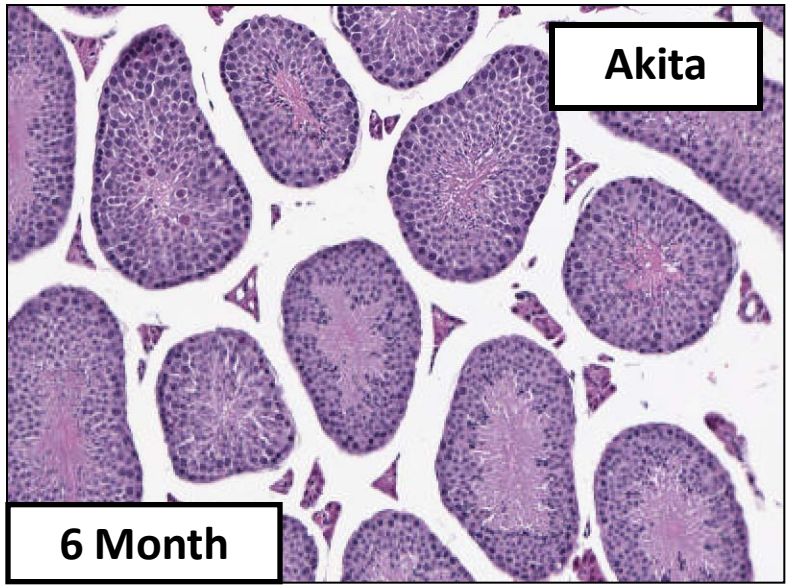
   (M. Toyoshima et al., 2007, have shown that gonadectomy of Akita mice alters expression of appetite regulating genes.)

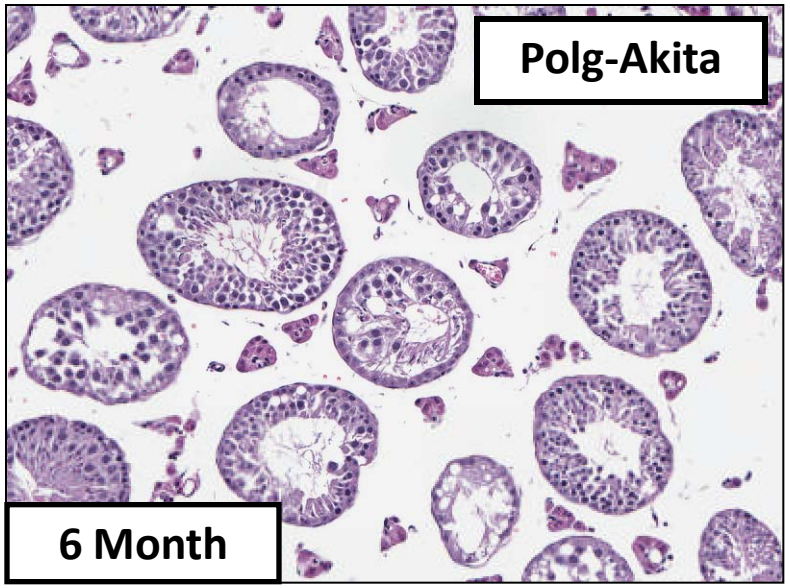
Proliferation

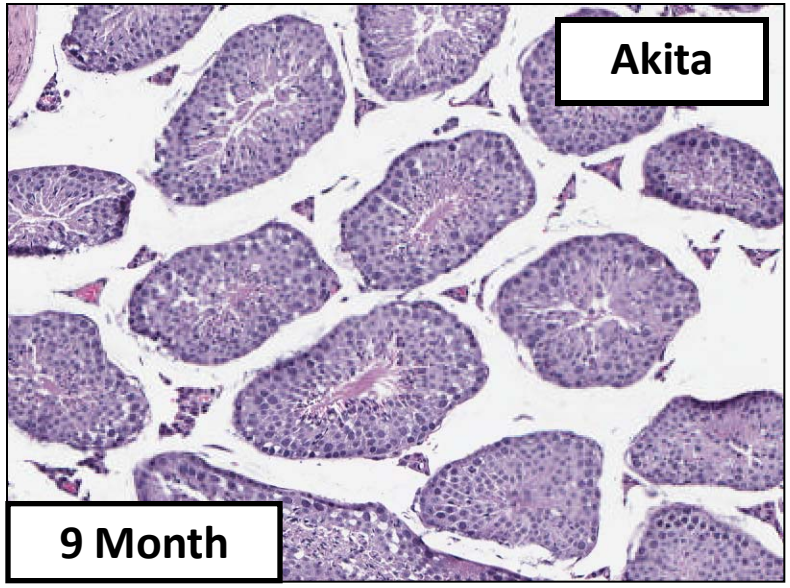


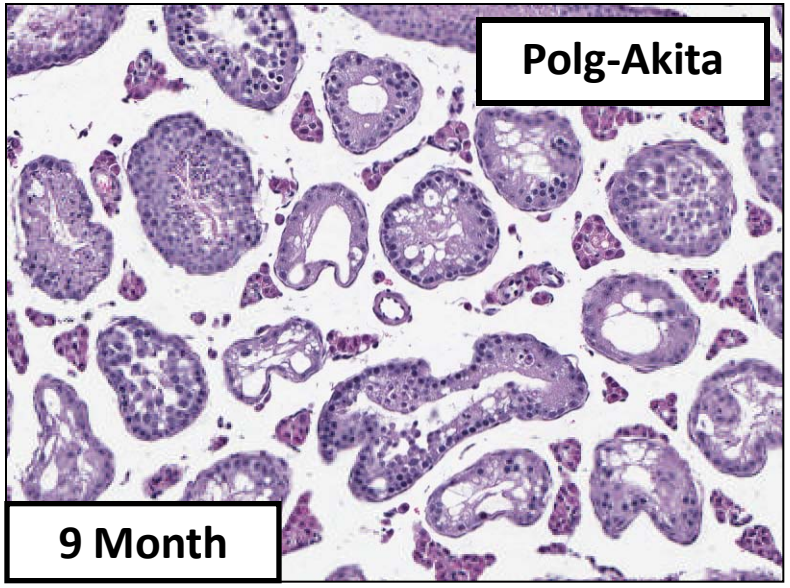
Apoptosis


DAPI (DNA)
TUNEL Assay
Merge
Akita
Akita
Akita
Polg-Akita
Polg-Akita
Polg-Akita

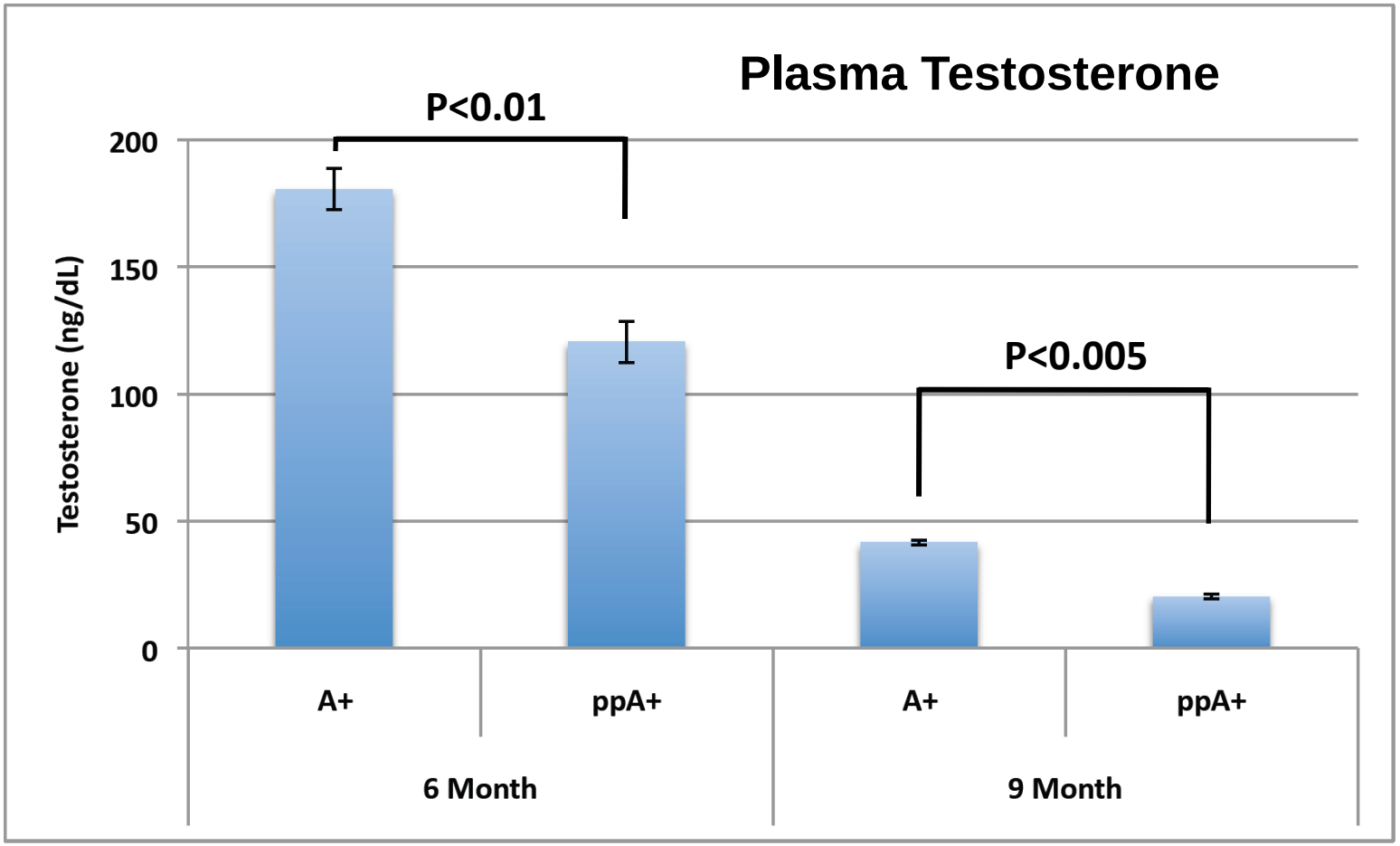

Akita
6 Month


Polg-Akita
6 Month

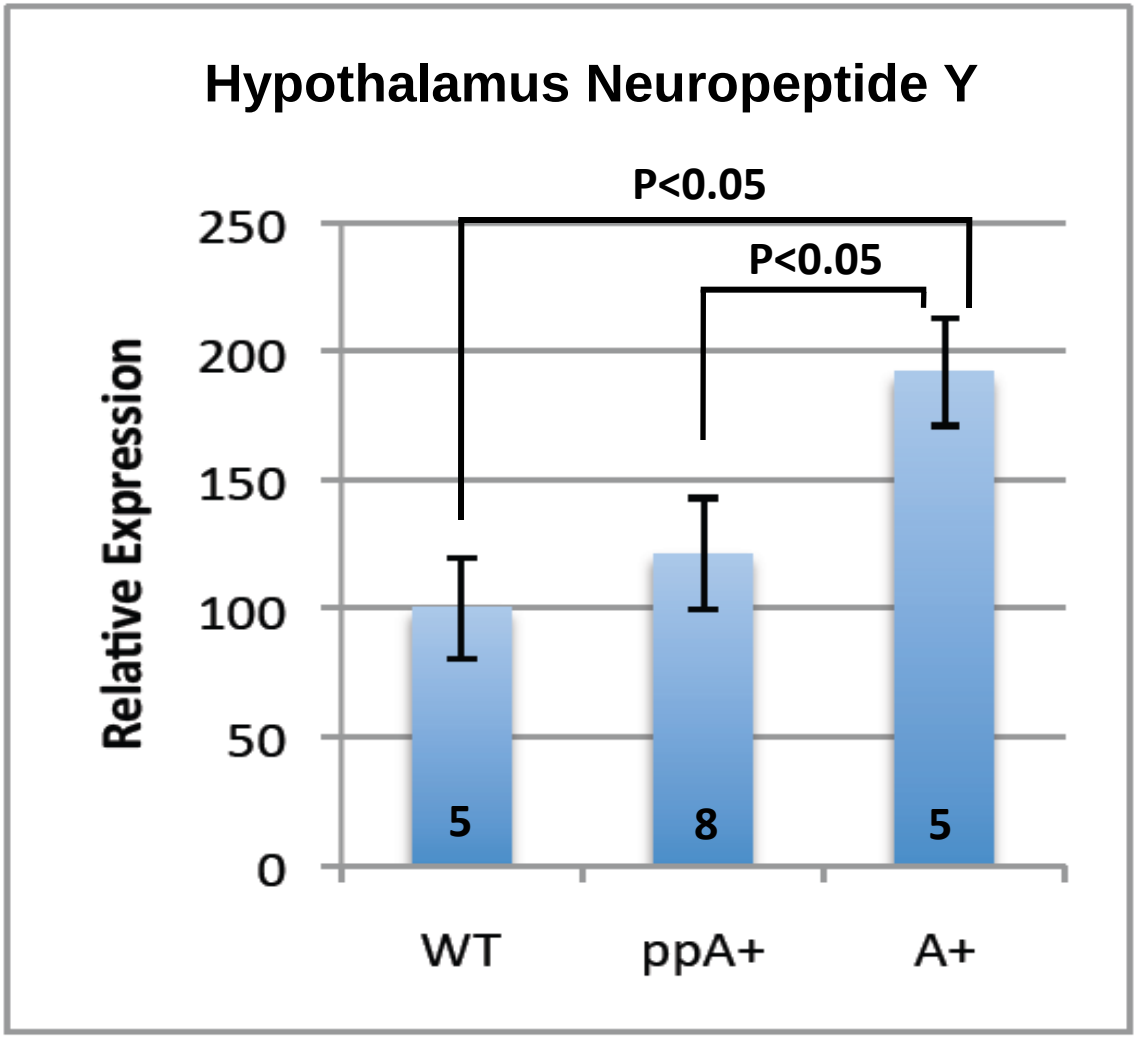

Akita
9 Month


Polg-Akita
9 Month

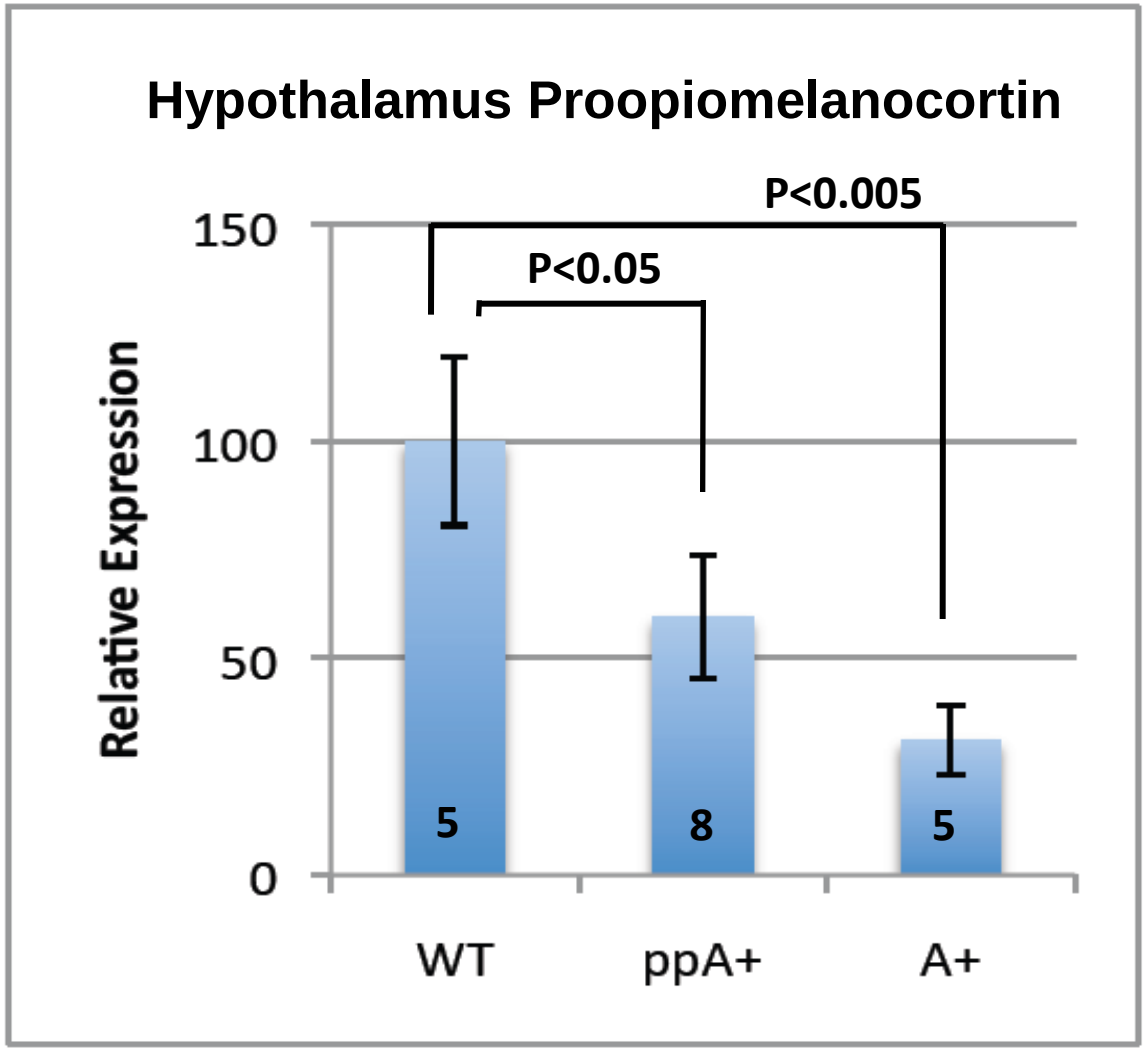
General Testis Function



Appetite-Stimulating Gene

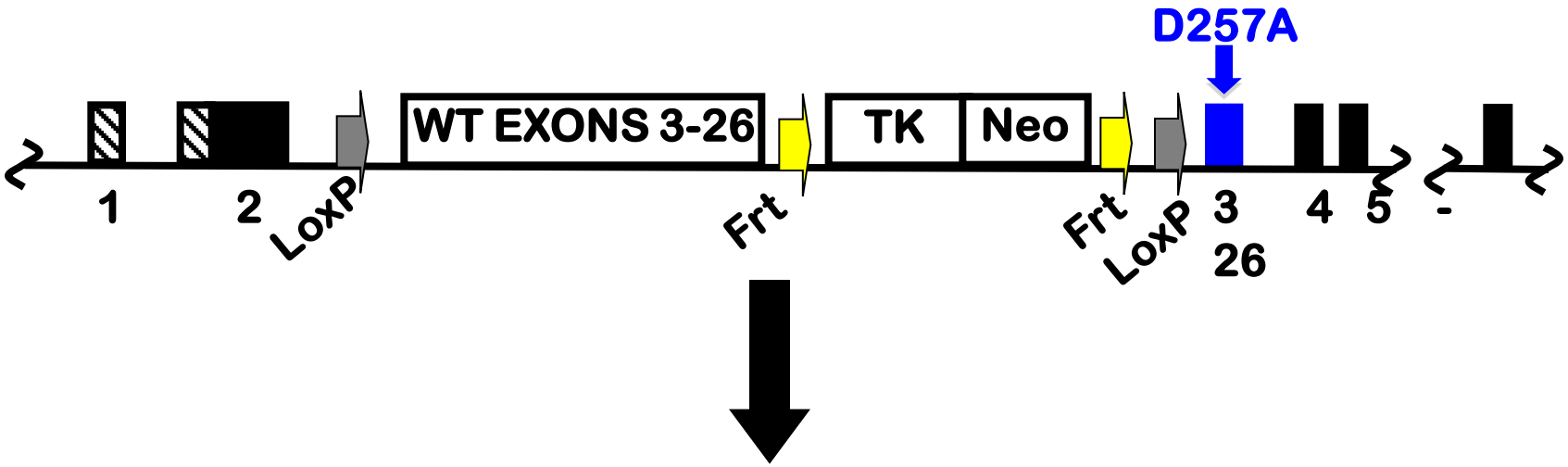


Appetite-Depressing Gene



Conclusions

- Intestinal dysfunction reduces nutritional uptake, while testicular dysfunction reduces food intake, both of which lessen the diabetic phenotype.
- The nutritional effects of the global *Polg* mutation are so great that uncovering the effects on diabetic nephropathy of renal mtDNA mutations using the global mutation is likely to be very difficult.
- We are therefore generating a conditional system to evaluate the tissue specific effects of mtDNA mutations.
- We will also transplant *Polg* mutant kidneys into Akita diabetic recipients.



Good chimeras,
but no transmission

Reinjecting with
different ES cell