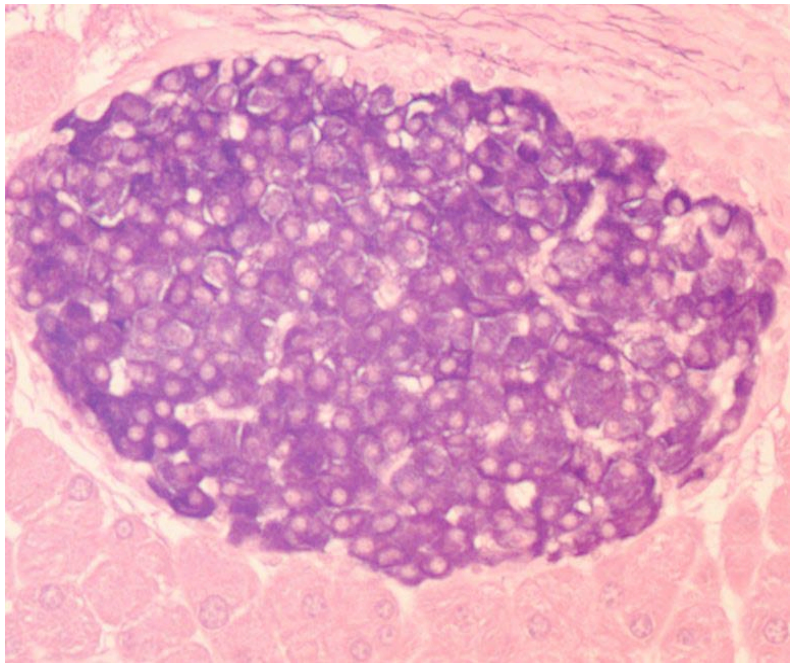
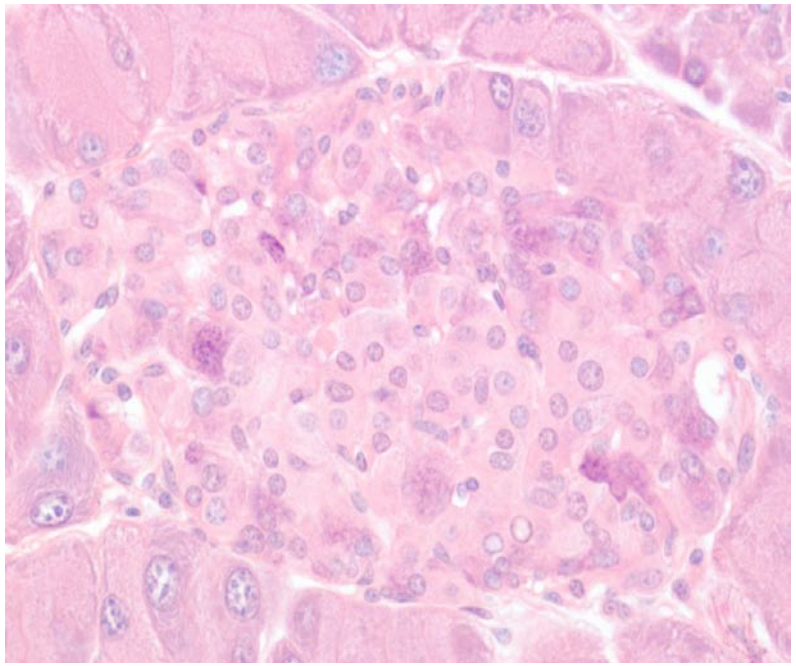
AMDCC Phenotyping at JAX

Aldehyde fuchsin-stained islet in 25-wk-old FVB-+/+ female pancreas



Aldehyde fuchsin-degranulated islet in 25-wk-old FVB-Akita/+ male pancreas



AMDCC

The Jackson Laboratory

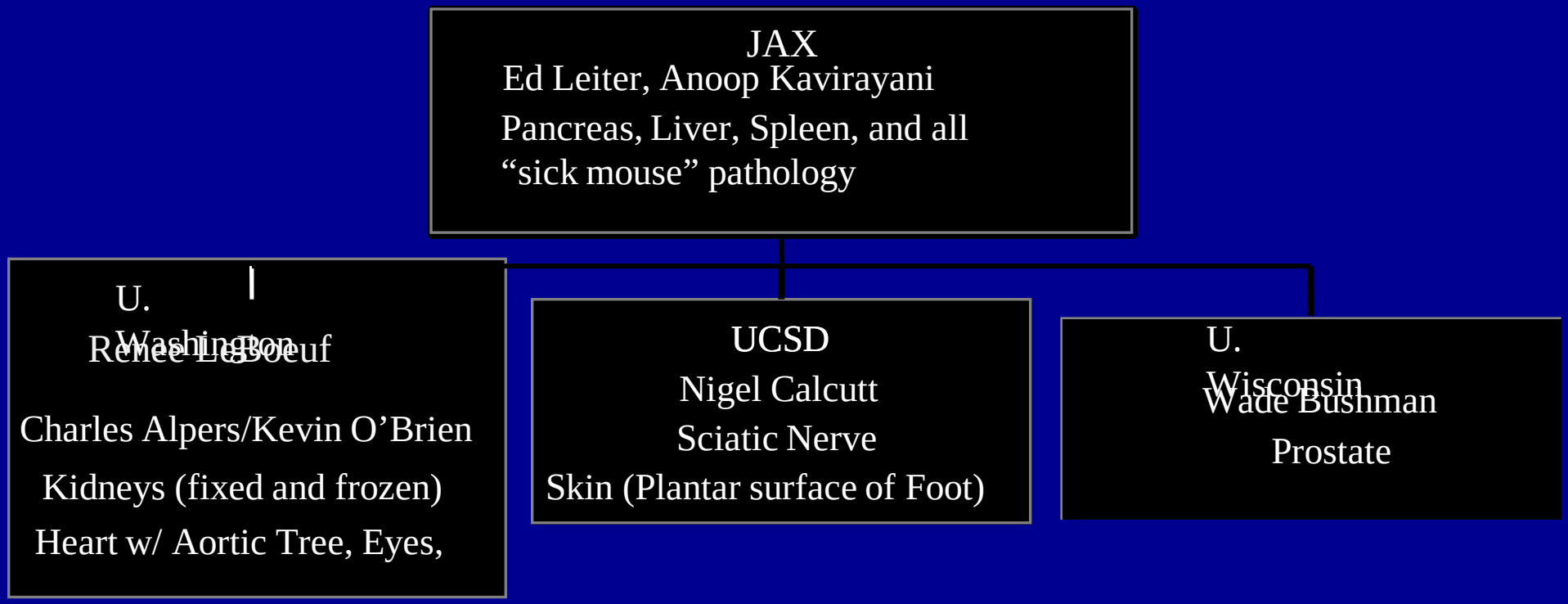
Models Phenotyped

Stock	Completed	In Progress	Other
FVB-Lepr^{db}	√		
FVB-Ins2^{Akita}	√		
DBA/2-Ins2^{Akita}	√		
B6-Ins2^{Akita}	√		
B6- Bdkr1/2 KO Ins2^{Akita}	√		
FVB-FXR KO Ins2^{Akita}		√	
B6-Ldlr KO Ins2^{Akita}		√	
129/SvEvTac Ins2^{Akita}		√	
129B6F1-eNos KO Ins2^{Akita}		√	
B6-eNos KO Ins2^{Akita}		√	
FVB-OVE26 (Rip-Calmodulin)			√
KK-Ins2^{Akita}			√

Phenotyping (JAX)

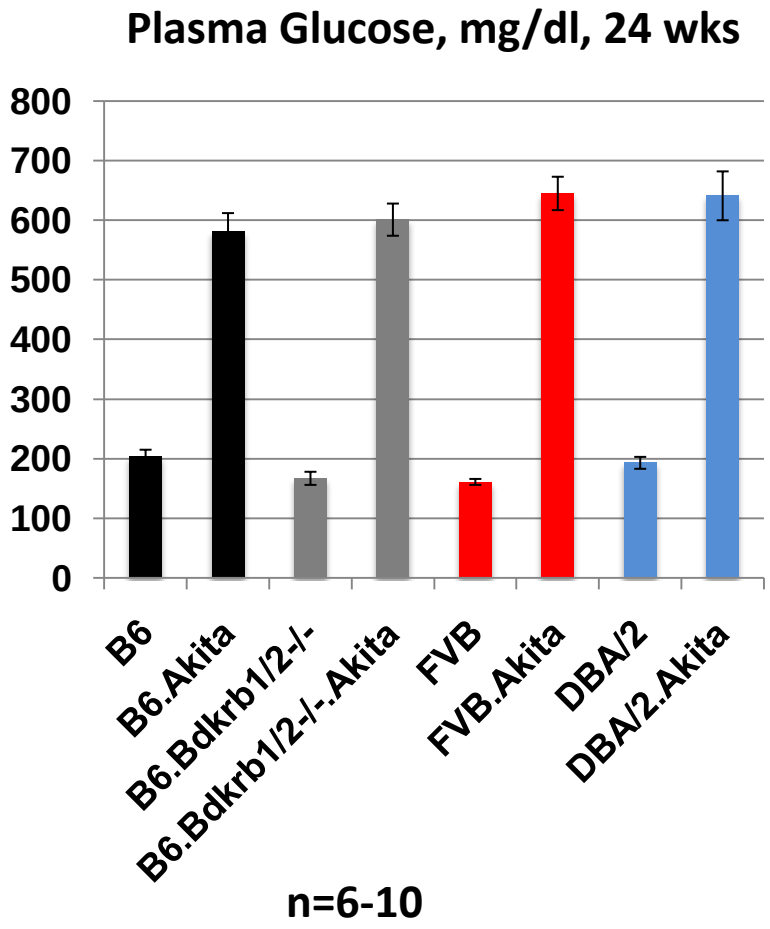
- Mice housed 2-5 in double-pen PIV racks, cob bedding w/nestlets, 11% w/w fat diet (LabDiet, 5LA6), acidified water, 12/12 light/dark cycle.
- Body Weight: 4-24 wks, every 2 wks
- Plasma Glucose (Beckman, Glucose Analyzer2) and Hematocrit: 4-24 wks, every 4 wks
- Plasma Insulin (Crystal Chem, ELISA): 8, 16, and 24 wks
- Pancreatic Insulin Content at 25 wks
- Electrocardiogram (Mouse Specifics, AnonyMOUSE): 13 and 21 wks
- Blood Pressure (Visitech Systems, BP-2000): 19 wks
- Albumin/Creatinine Ratio (Exocell, ELISA): Spot Urine at 17, 21, and 25 wks, 24-hour Urine Collection Cages (Nalgene/Tecniplast) at 21 and 25 wks
- Dual Energy X-ray Absorptiometry (DEXA, Lunar PIXImus2): 25 or 26 wks
- Spleen, Kidney, and Brain Tissue Weights: 26 wks
- Plasma Chemistry (Beckman Synchron V): 26 wks (Glucose, GLDH, BUN, TC, HDL-C, TG, and NEFA)

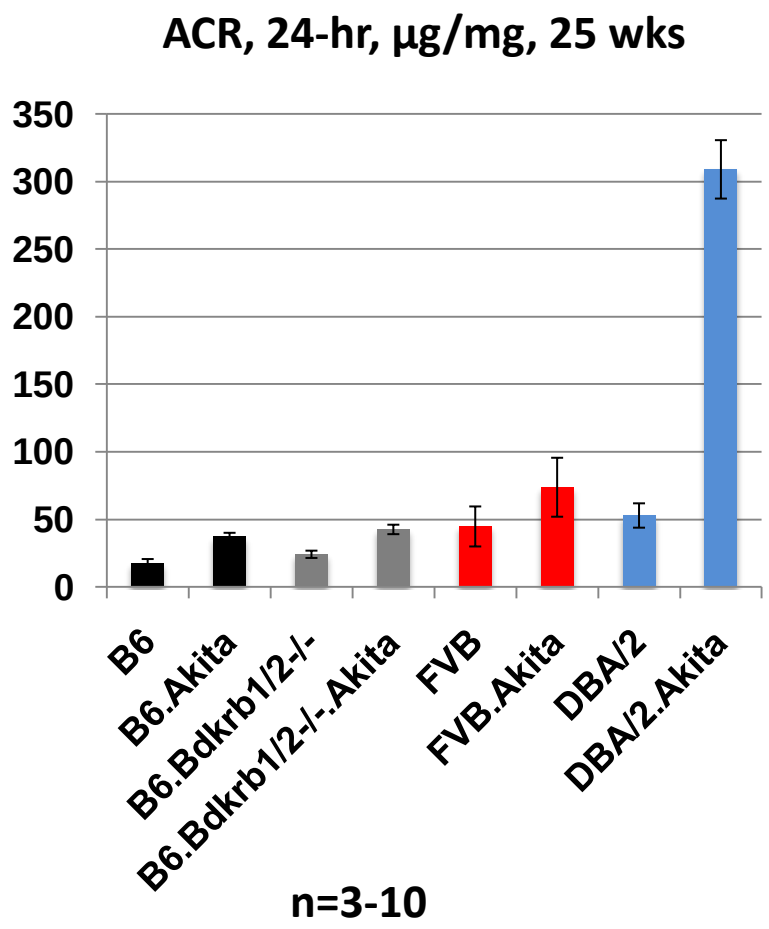
Extramural Histologic Assessment
(saline perfusion in JAX Necropsy Service, NBF) fix



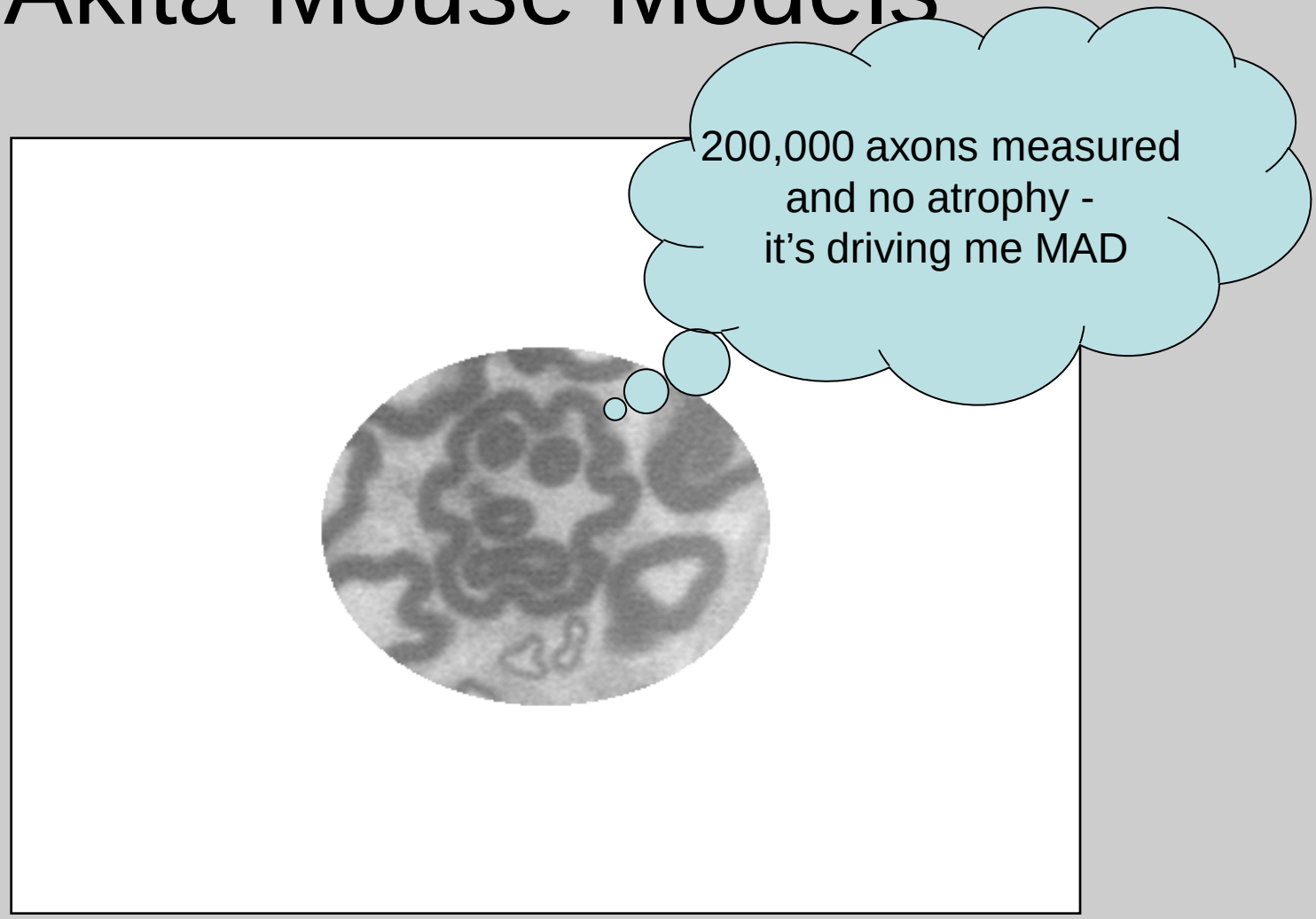
Diabetes phenotypes are robust, but diabetic nephropathy associated phenotypes are attenuated

(data for 25-wk-old males)





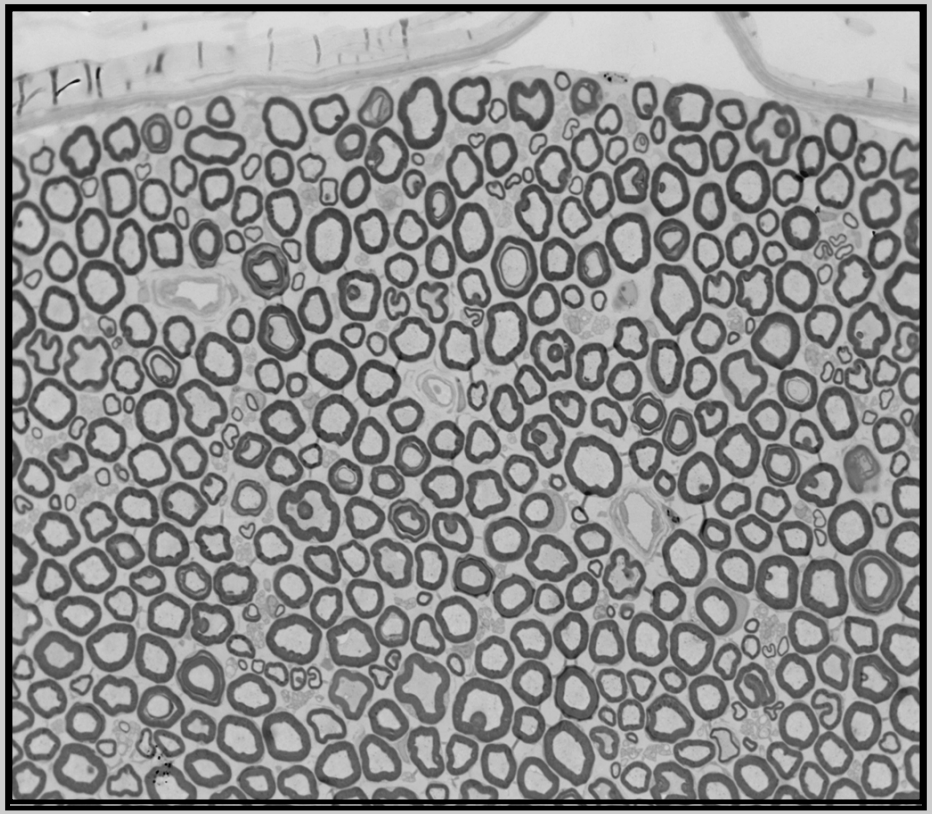
Neuropathy Phenotyping in Akita Mouse Models



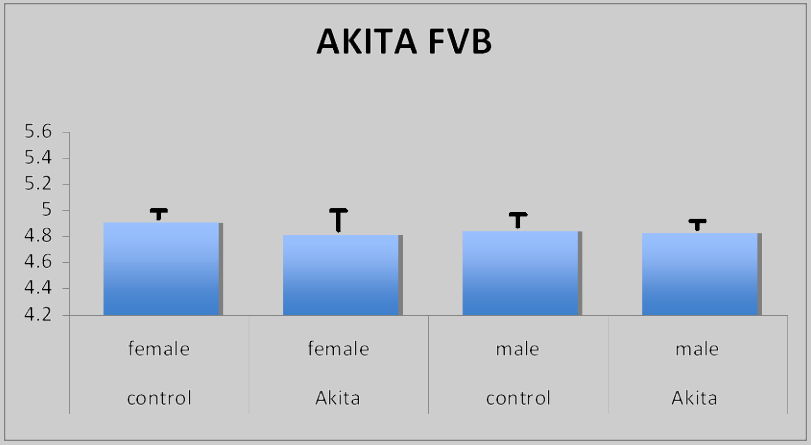
Nigel Calcutt, Dept of Pathology, USCD

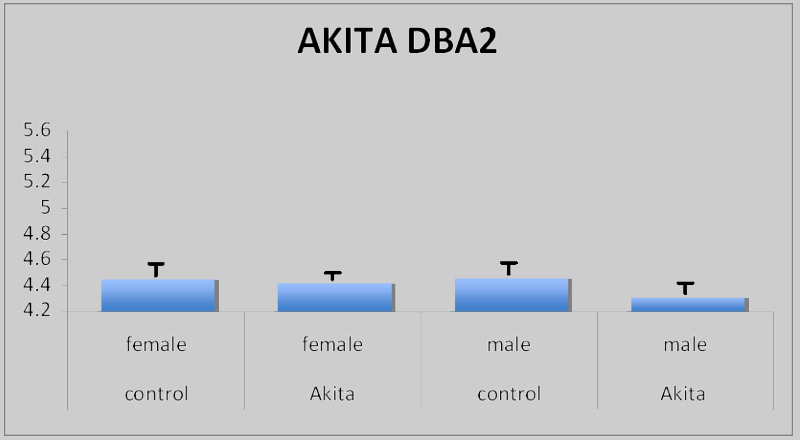
Sciatic Nerve Morphometry

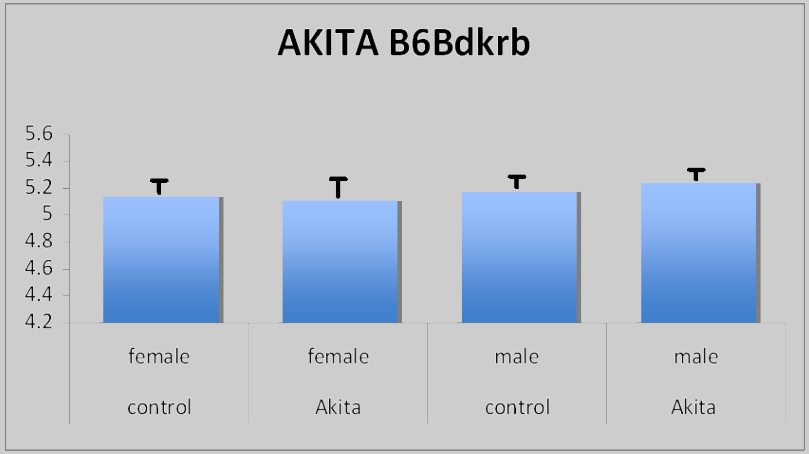
- Sciatic nerve immersion fixed, stored in buffer and shipped to UCSD
- Tissue processed to plastic and 1 µm sections cut for light microscopy and morphometry using Scion Image program
- Nodal and paranodal profiles excluded before measuring axonal diameter of approximately 1,500 axons per nerve
- Mean axonal diameter (MAD) calculated
- Axons are also sorted into incremental 1 µm bins to allow assessment of size:frequency distribution
- We are measuring diameter of myelinated motor and sensory fibers, the largest of which are those that provide the NCV measurement
- Reduced MAD contributes to NCV slowing in long term STZ-diabetic rats

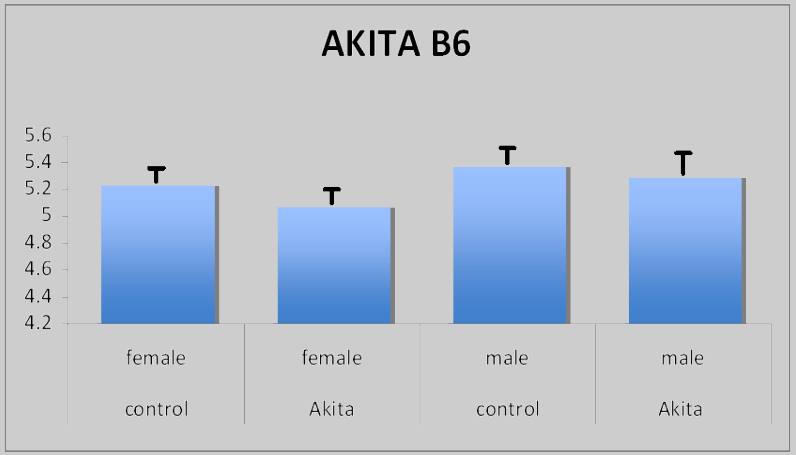


MAD in Akita Mice





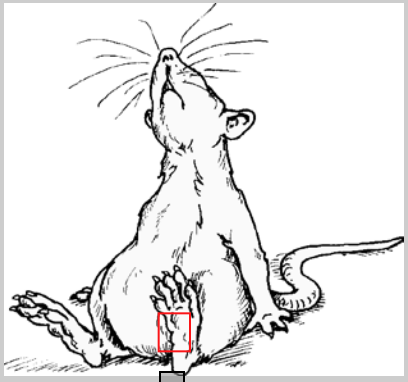


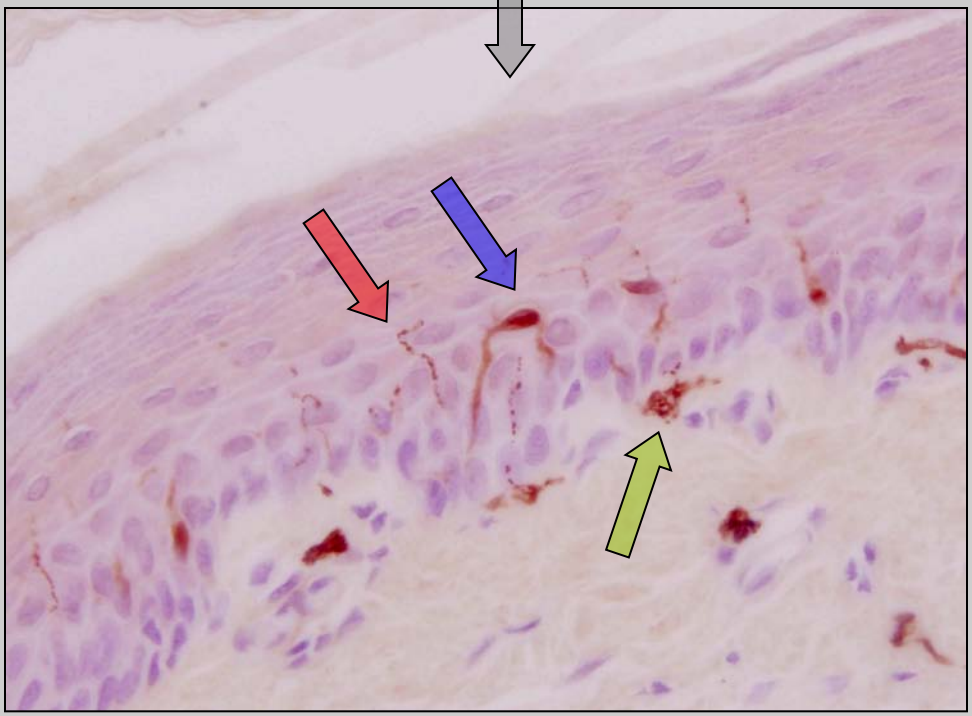


- No change in MAD (µm) or fiber frequency distribution (not shown) in any strain.
- In other recent studies we have also found no sciatic MAD reduction in either Akita (Bl/6) db/db, STZ (C57Bl/6) or STZ (Swiss Webster) mice.

Nerve Terminals in Skin

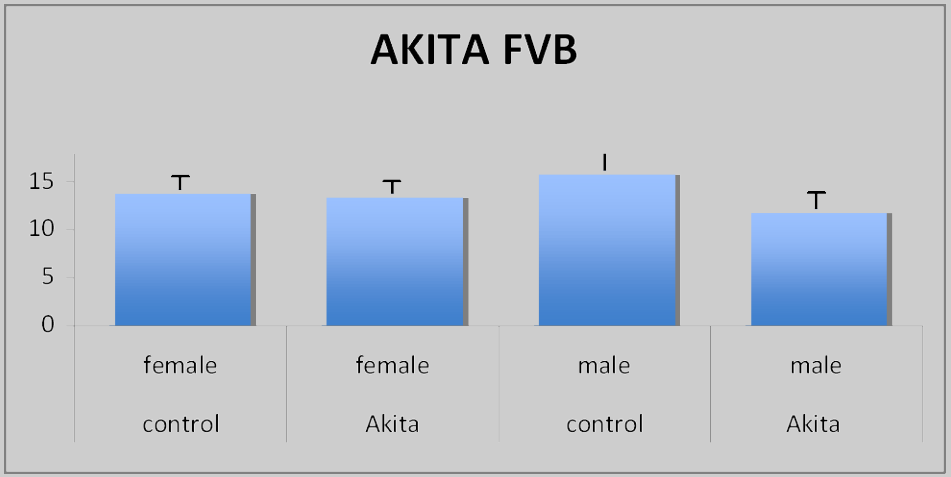
- Plantar foot skin fixed, stored in buffer and shipped to UCSD
- Skin samples immunostained with anti-PGP9.5 to identidy intra-epidermal nerve fibers (IENF), sub-epidermal nerve profiles (SNP) and Langerhans' cells
- Immunoreactive profiles of IENF (red), SNP (green) and Langerhans' cells (blue) counted and normalized against length of the dermal:epidermal border
- IENF are unmyelinated fiber terminals that sense temperature and which are lost in diabetic patients and rodents, leading to thermal hypoalgesia

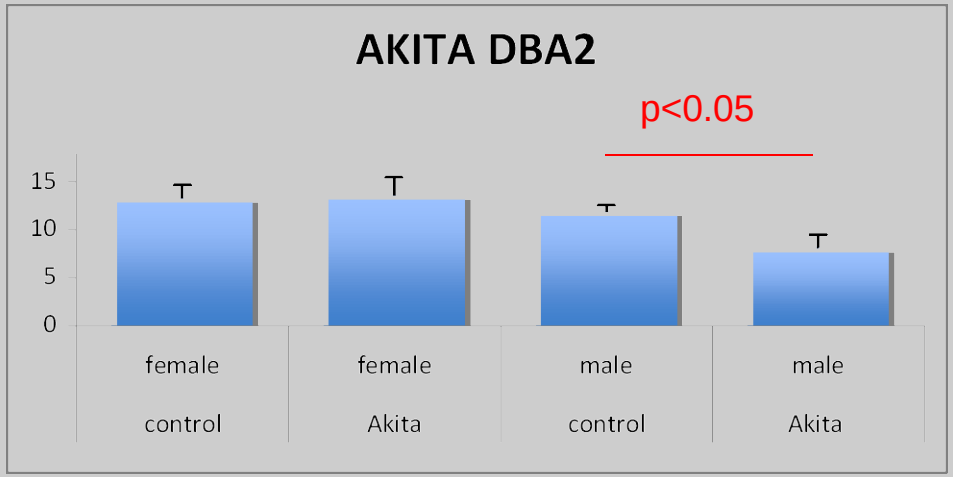


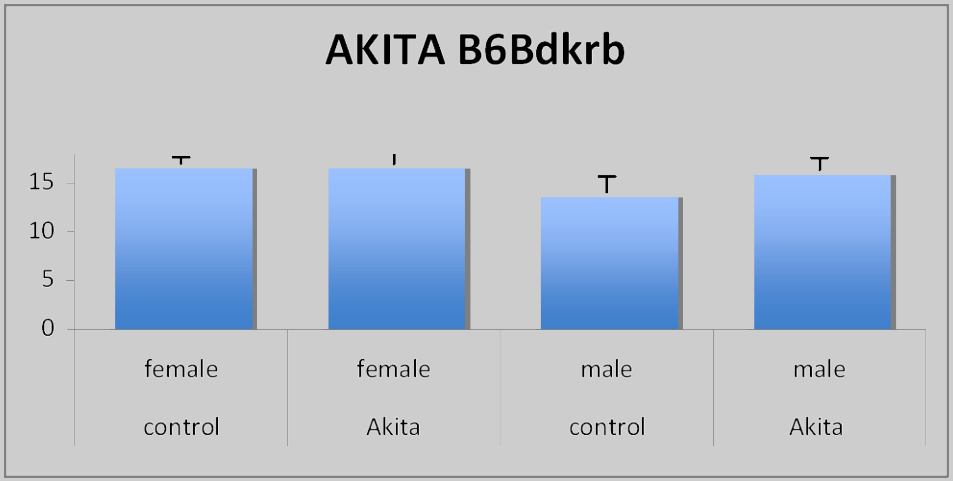


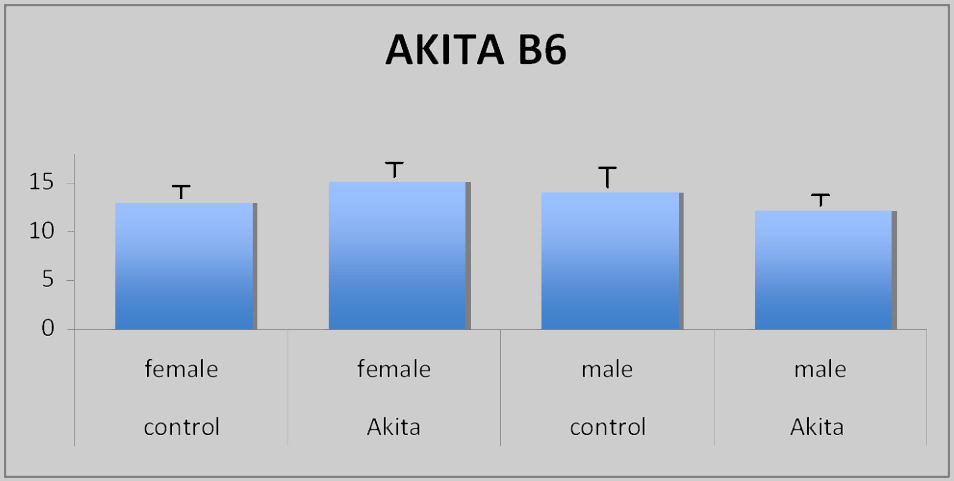
100x

Skin





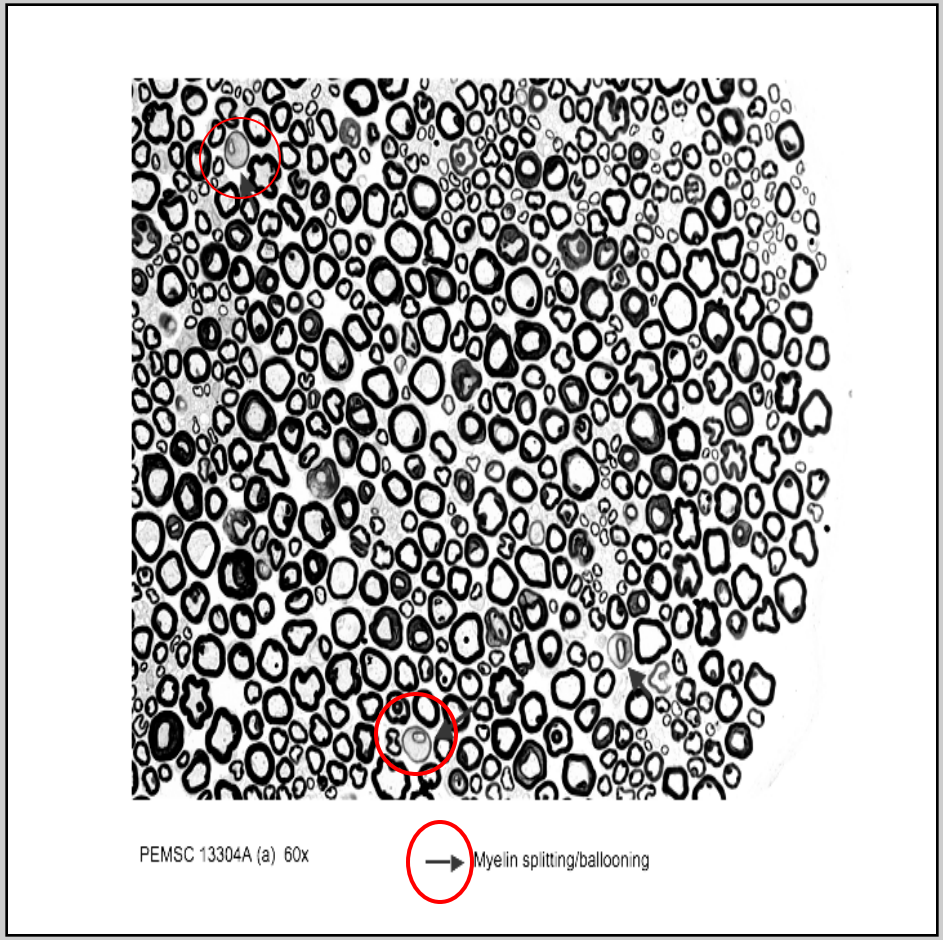


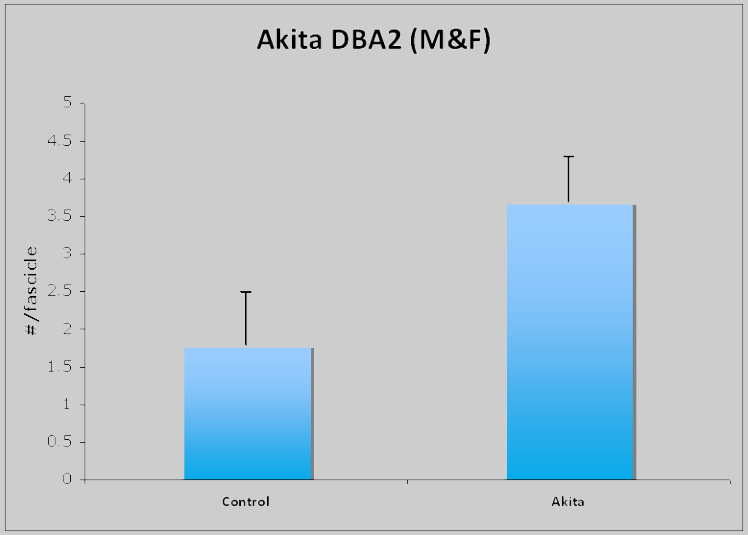


- IENF loss in MALE Akita DBA2 and trend in MALE Akita FVB
- No loss of SNP or change in Langerhans' cells (not shown)
- We have previously seen IENF loss in db/db (C57Bl/6), STZ (C57 Bl/6), STZ (Swiss Webster) mice after 4 weeks + of diabetes

Bonus Data

- Occasional myelin splitting noted in sciatic nerve sections from Akita DBA2 mice
- Blinded re-count shows significant increase in both males and females
- Not seen in other Akita strains evaluated to date.
- Measurement of myelin thickness in Akita DBA2 in progress





Summary

- No clear indices of structural neuropathy in male or female Akita FVB, B6 or B6-Bdkrb KO mice.

- Male (not female) Akita DBA/2 mice show significant but mild (~30%) IENF loss and occasional myelin splitting.

- Reduced MAD in sciatic nerve not seen in any mice or any other type of diabetic mouse we have examined:
    - May be a rat thing
    - Nerve pathology is reported in more distal nerves of STZ-mice so perhaps we need to start collecting sural/tibial nerves

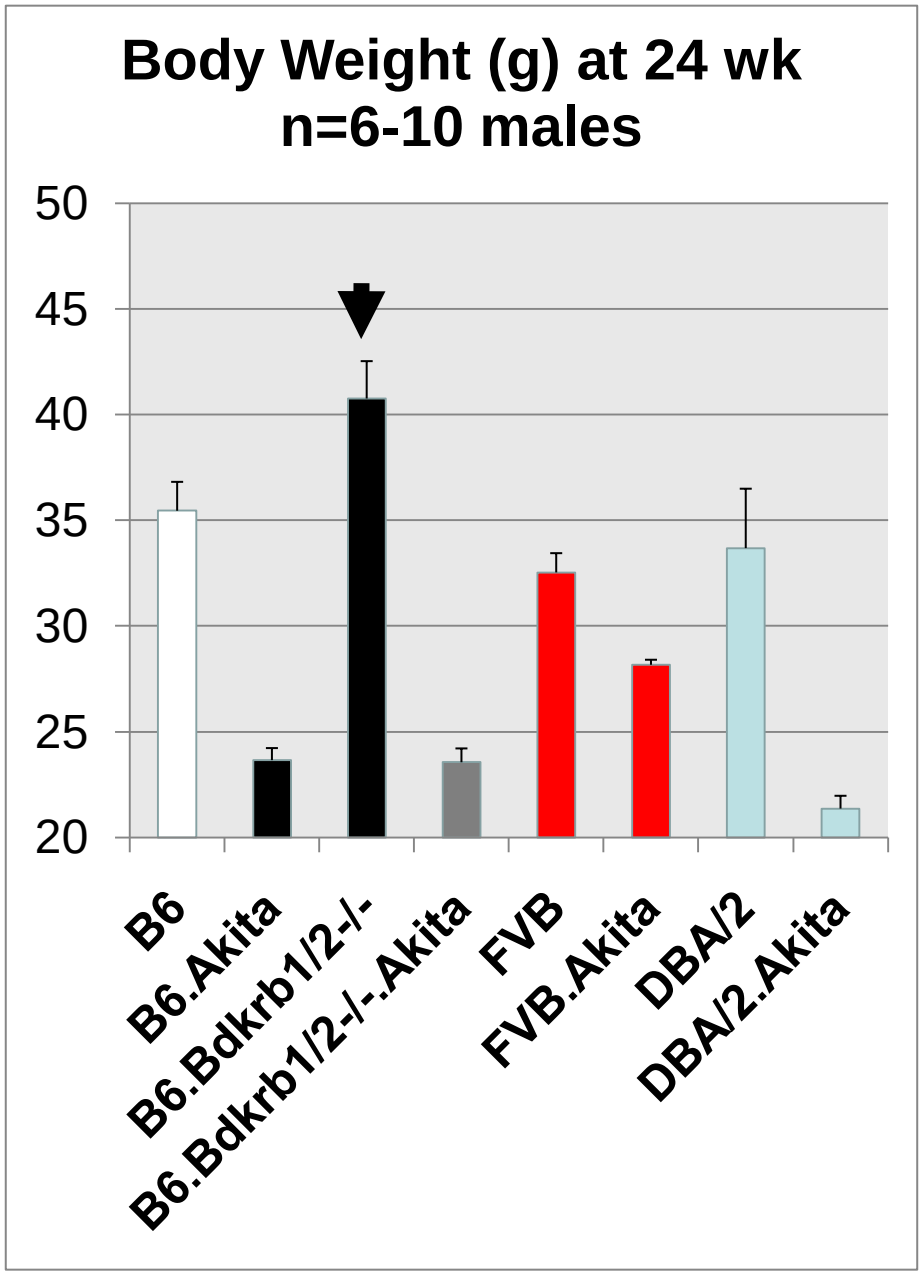
- At UCSD, in 25 separate studies since 2005, more dramatic (>50%) IENF and SNP loss has been seen in STZ mice (4wk+) on many backgrounds and also in long-term (insulinopenic) db/db (C57Bl/6) mice.
    - We suspect that IENF loss requires both hyperglycemia and marked insulinopenia as minimal insulin supplementation can maintain normal IENF levels in STZ rats and mice.
    - Insulin status of Akita mice?

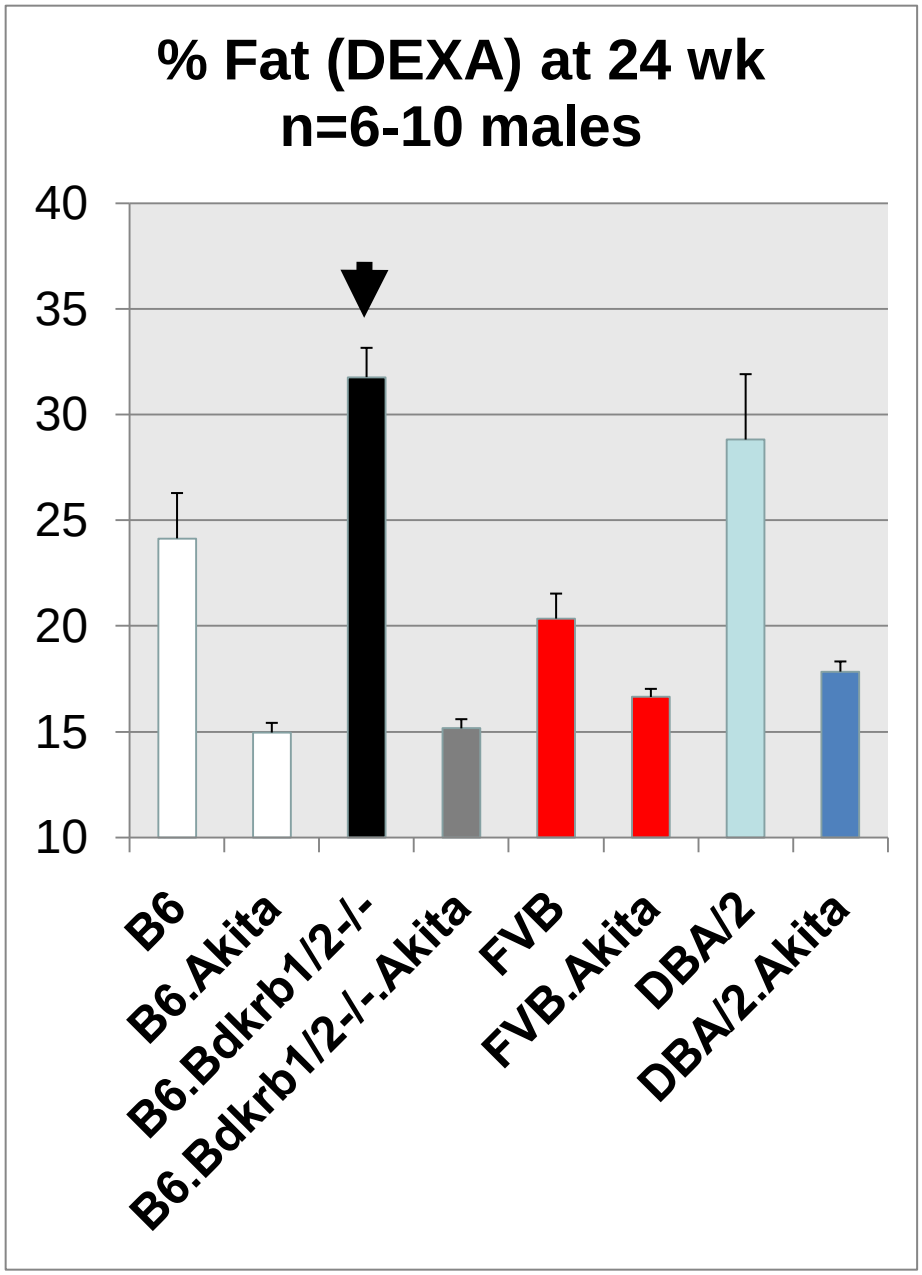
Why are AMDCC stocks maintained at JAX proving more resistant to complication pathologies?

Items for consideration.

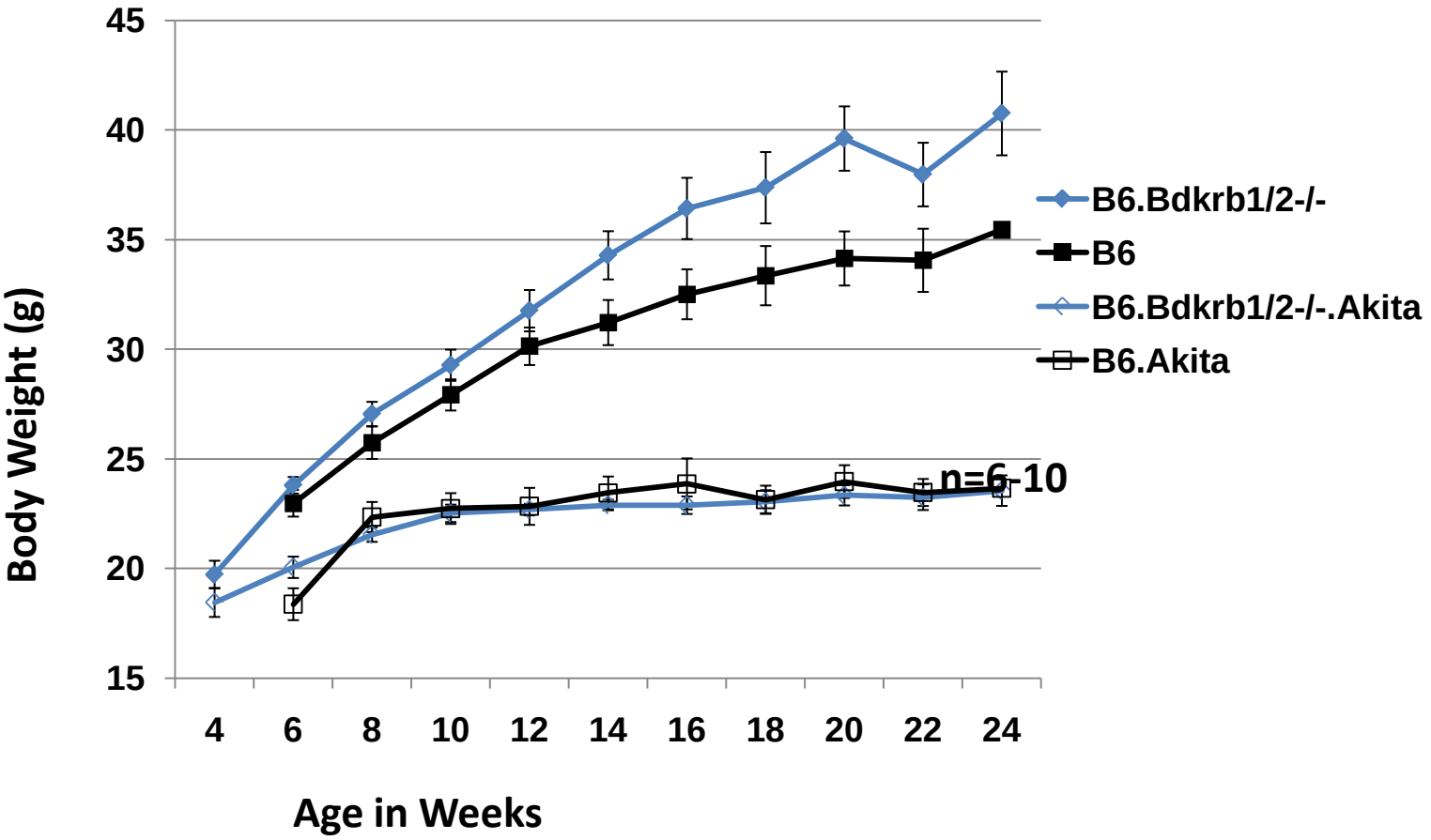
- Differences in diet? JAX uses a 10-11% fat (w/w) containing chow (Research Diets 5LA6). This “breeder” chow may render diabetic Akita mice and other KO stocks more robust.
- Other husbandry differences?
- Substrain differences?
- Microbiota differences?
- Physiologic/metabolic differences? Residual insulin? Direct toxicity of STZ? Rats vs. mice?

Indications of B6 Substrain Differences





Males of Two B6 Strains on 11% Fat Chow Diet 5LA6



Both murine substrain and microbiome can interact to influence phenotype

Specific Microbiota Direct the Differentiation of IL-17-Producing T-Helper Cells in the Mucosa of the Small Intestine

Ivaylo Ivanov Ivanov, Rosa de Llanos Frutos, Nicolas Manel, Keiji Yoshinaga, Daniel B. Rifkin, R. Balfour Sartor, B. Brett Finlay, and Dan R. Littman

Cell Host Microbe (2008). 4:337-349.

Fig. 6: C57BL/6 Mice from Jackson Laboratory Are Deficient for Th17 Cell-Inducing Microbiota

10-wk-old

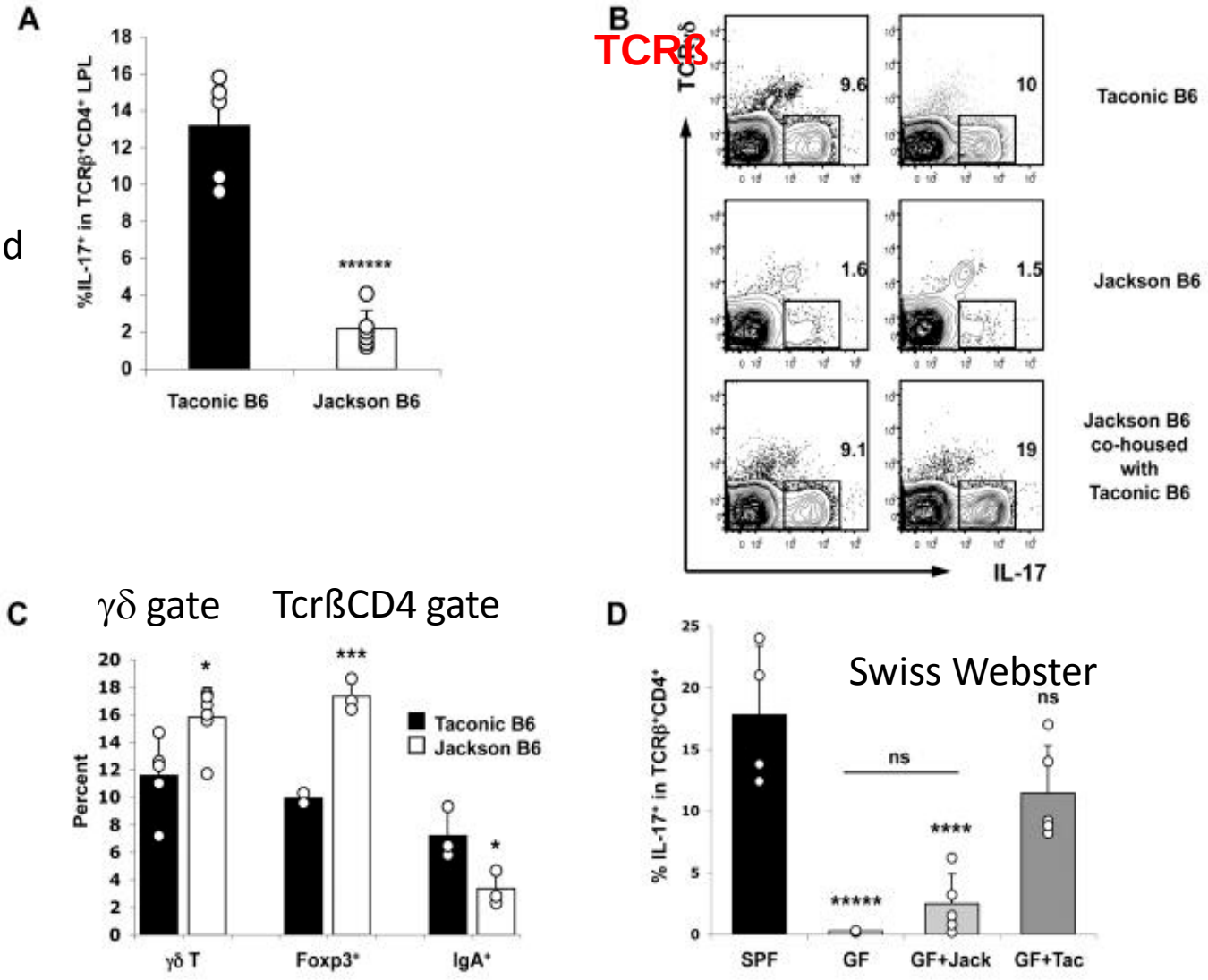
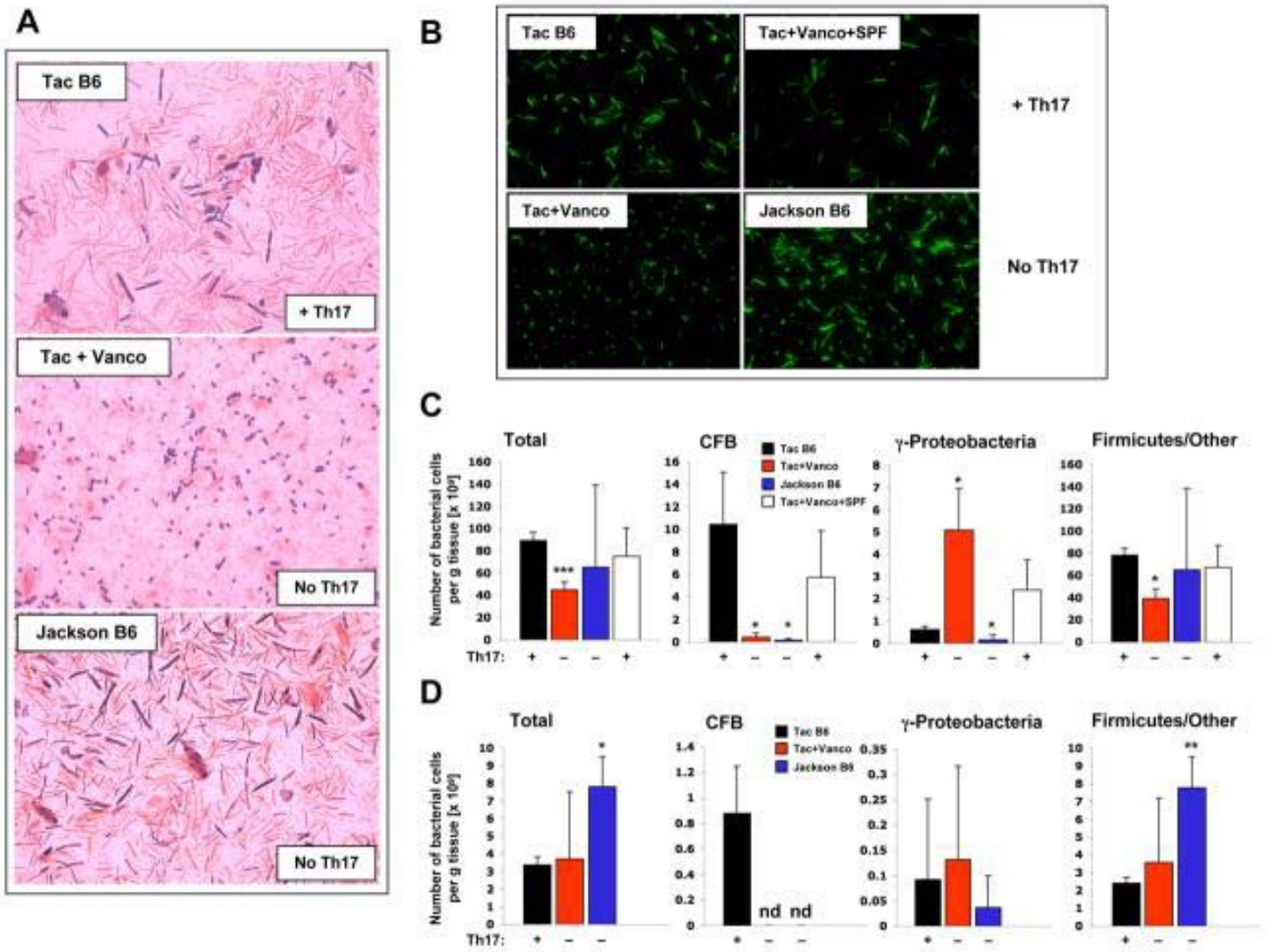


Fig. 7: Bacterial Composition in Mice with and without SI LP Th17 Cells (CFB phylum=Gram-; Firmicutes=Gram+)



cecum

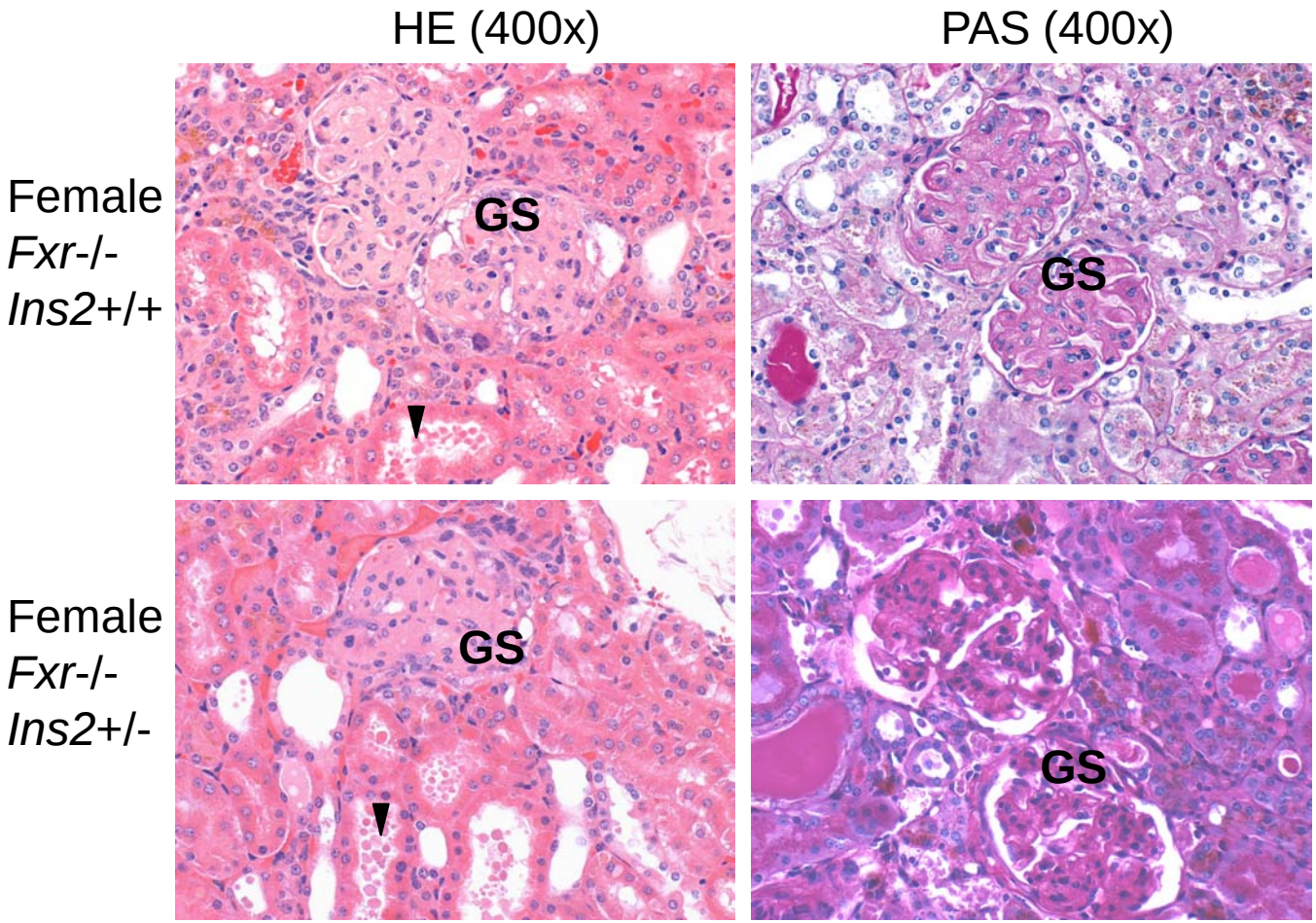
Term. ileum

What constitutes a diabetic complication versus a KO-generated pathology?

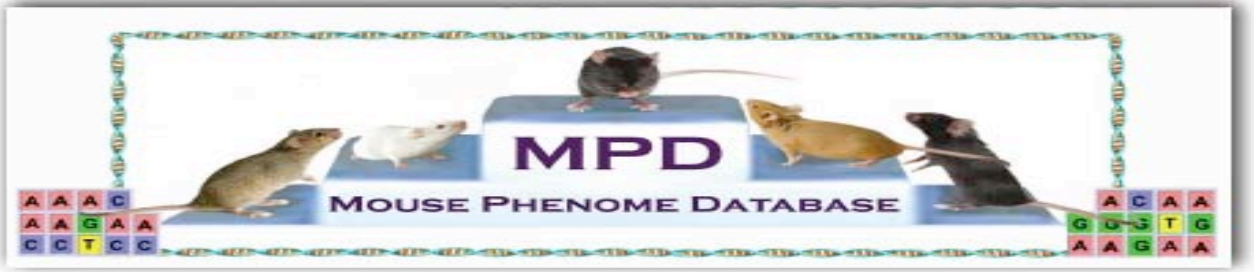
Case of the FXR KO on the FVB/N background.

We have recorded 6 deaths of FXR-/- female breeders from our FVB-FXR-/- Akita/+ breeding colony between the ages of 16-26 wk. Four of these FXR-/- females were wildtype at Ins2 (not Akita). Only one was an Akita/+ heterozygote. Genotype of one was indeterminate.

Histopathologic analysis revealed that FXR deficiency was producing severe kidney and hepatic lesions independent of diabetes elicited by the Akita mutation.



GS – Global glomerulosclerosis
Arrowhead – tubular droplets (presumably proteinaceous)



Mouse Phenome Database (MPD)

www.jax.org/phenome

Publicly available JAC Projects

Korstanje1 Korstanje R Aging study: Urine albumin and creatinine
- kidney – function

Mills1 Mills K Aging study: Chromosome instability and apoptosis
- cell and tissue damage – apoptosis
- cell and tissue damage – chromosome instability

Peters4 Peters LL Aging study: Blood hematology
- blood hematology – CBC--differentials
- blood hematology – CBC--platelets
- blood hematology – CBC--red cell parameters

Petkova1 Petkova S, Roopenian D Aging study: Peripheral blood leukocytes (PBL profiles)
- blood hematology – CBC--differentials
- immunity – peripheral blood

Seburn2 Seburn KL, Xing S, Burgess RW Aging study: Grip strength and gait analysis
- muscle – skeletal
- nervous system – neuromuscular function

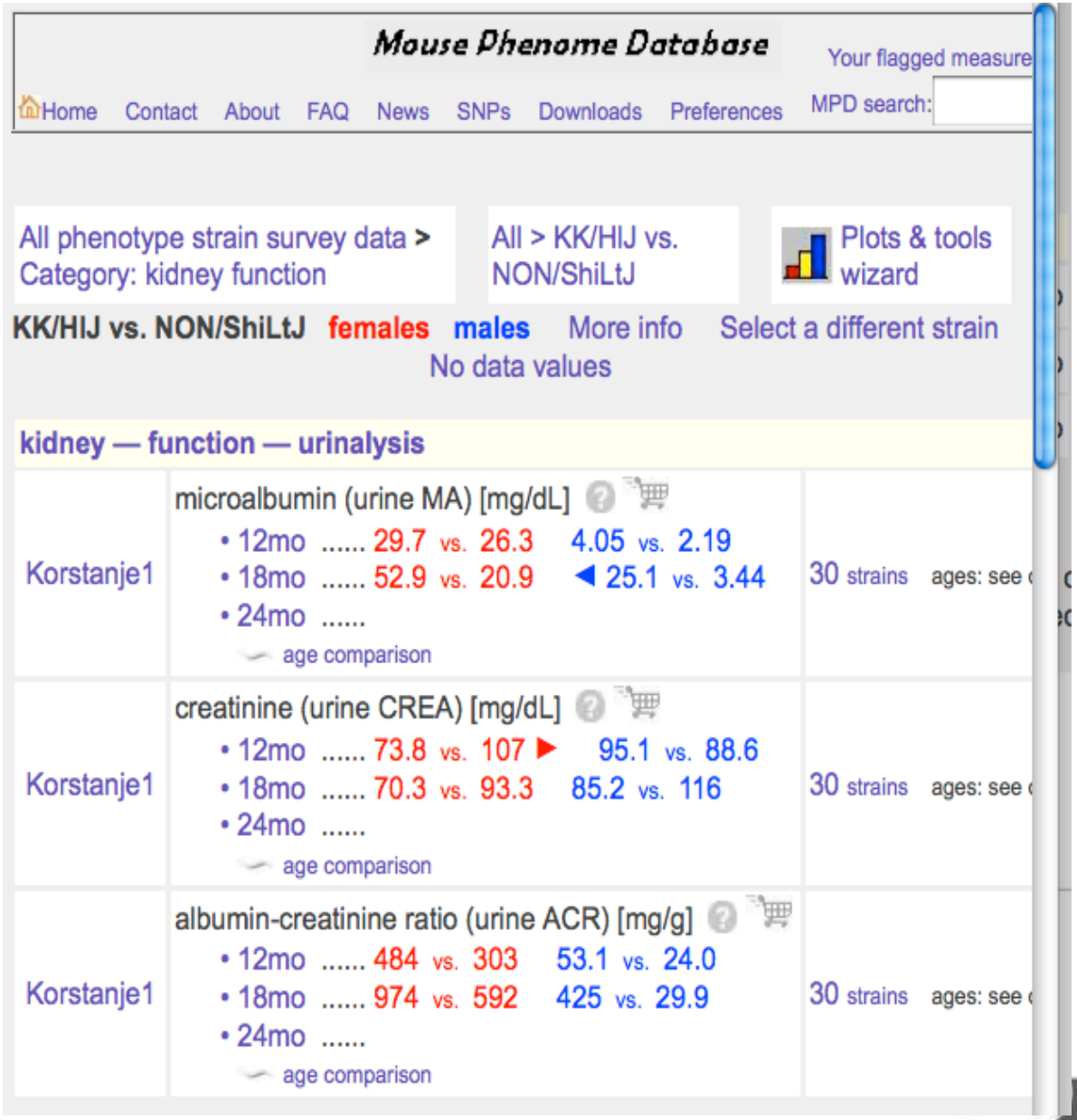
Sundberg1 Sundberg JP Aging study: Comprehensive histopathology
- cancer – tumor histopathology

Xing1 Xing S, Korstanje R, Paigen B Aging study: Electrocardiogram (ECG)
- cardiovascular – physiology and function

Yuan1 Yuan R, Rosen CJ, Beamer WG Aging study: IGF-1 and body weight
- body weight size and growth – body weight
- endocrine – hormones

Yuan2 Yuan R, Paigen B Aging study: Lifespan and survival curves
- longevity – survival curves

Yuan3 Yuan R Aging study: Blood chemistry
- blood chemistry – electrolytes
- blood chemistry – enzymes
- blood chemistry – miscellaneous
- blood chemistry – proteins
- blood chemistry – urea nitrogen
- blood lipids – cholesterol
- endocrine – hormones



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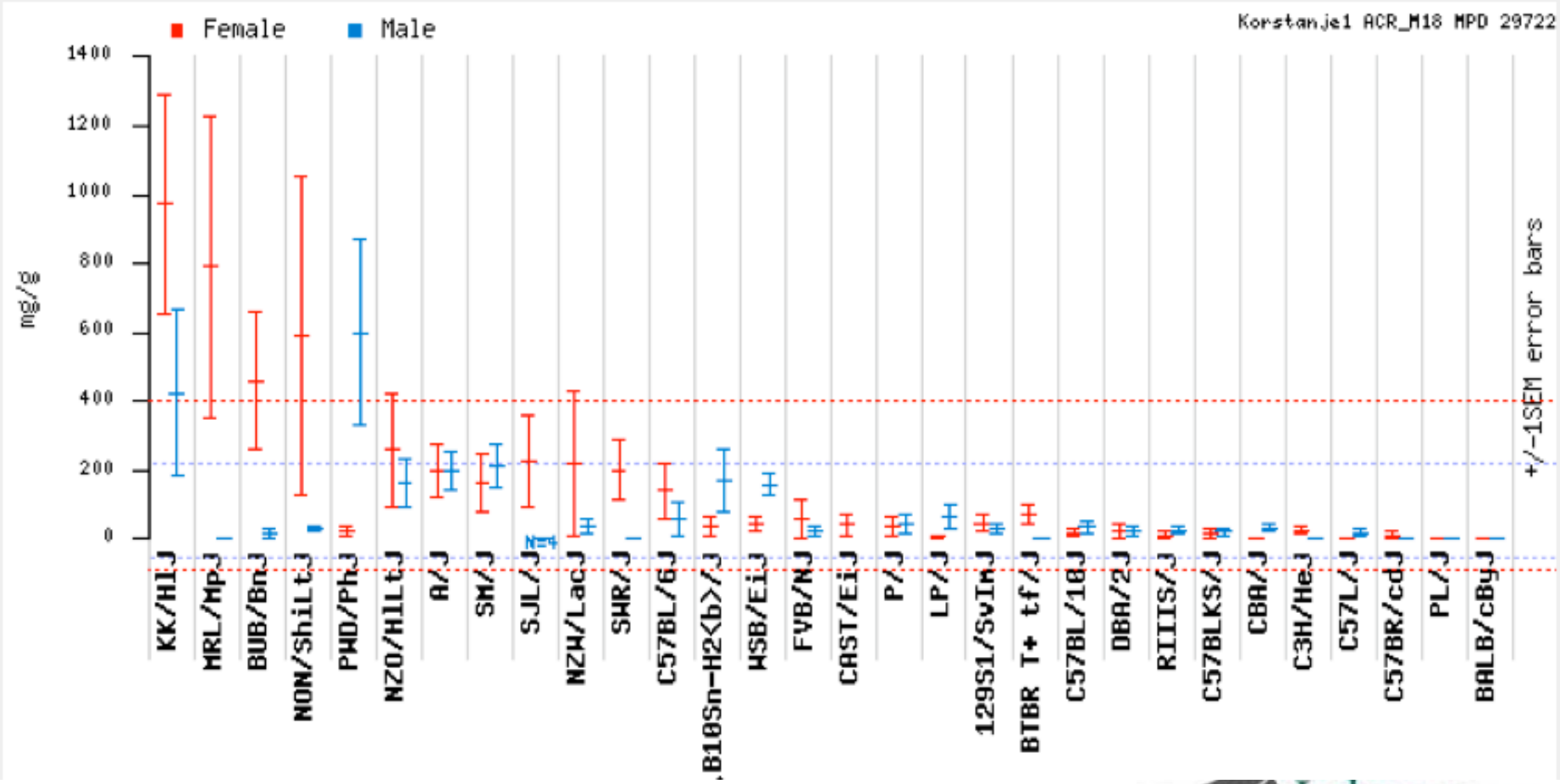
Measurement: ACR_M18

kidney — function — urinalysis

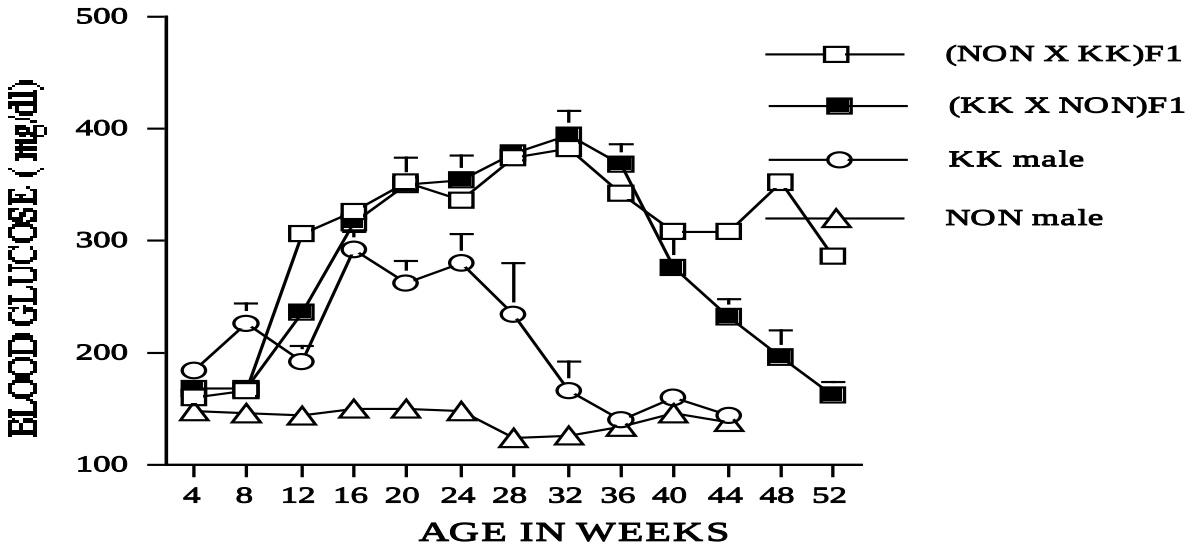
Korstanje1	albumin-creatinine ratio (urine ACR) [mg/g] • 12mo • **18mo** • 24mo age comparison	30 strains ages: see details

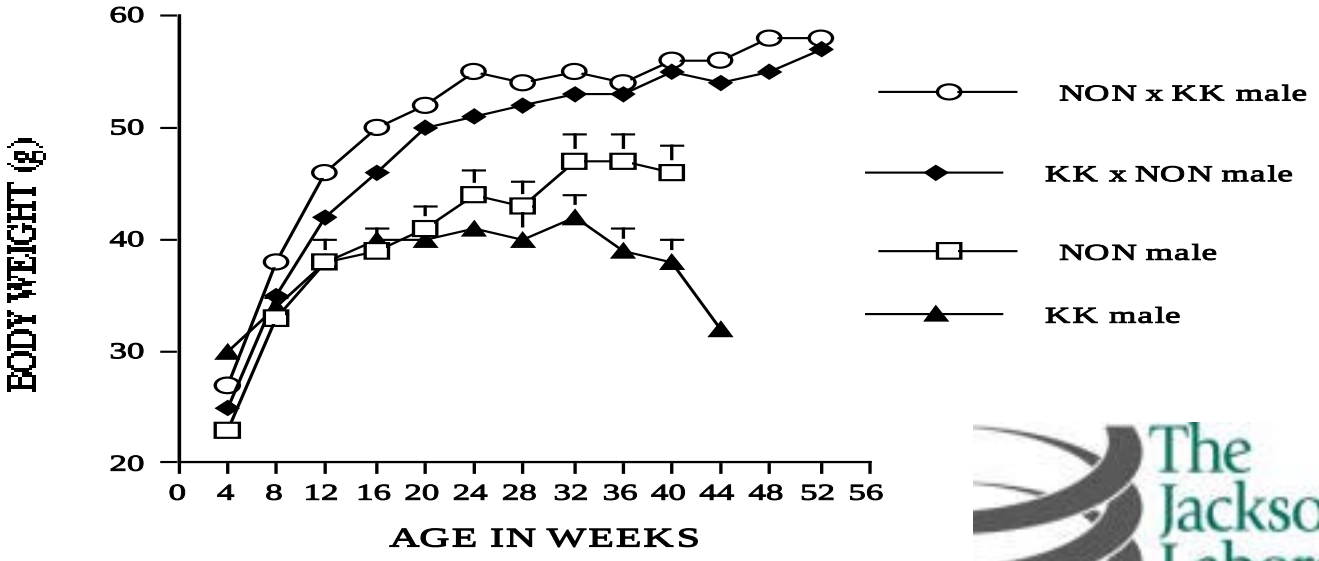
albumin-creatinine ratio (urine ACR) [mg/g]



Laboratory

NON Genome Exacerbates Diabesity in F1 Males





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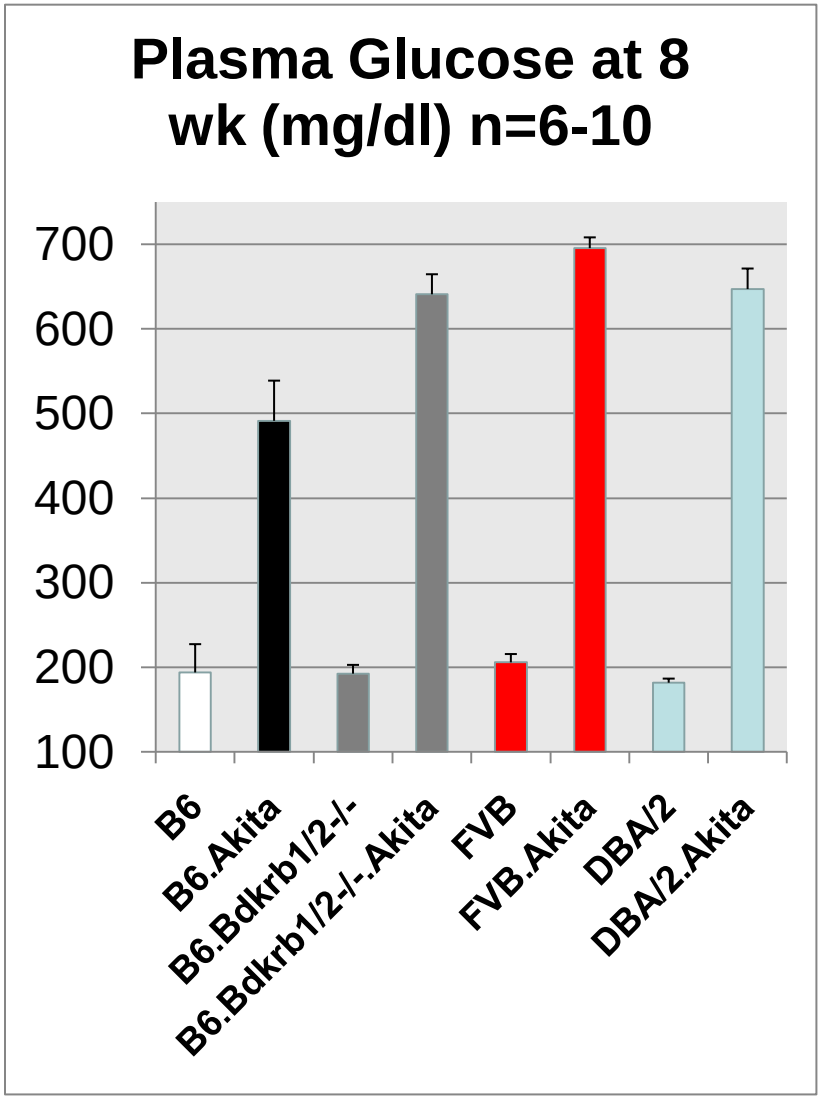
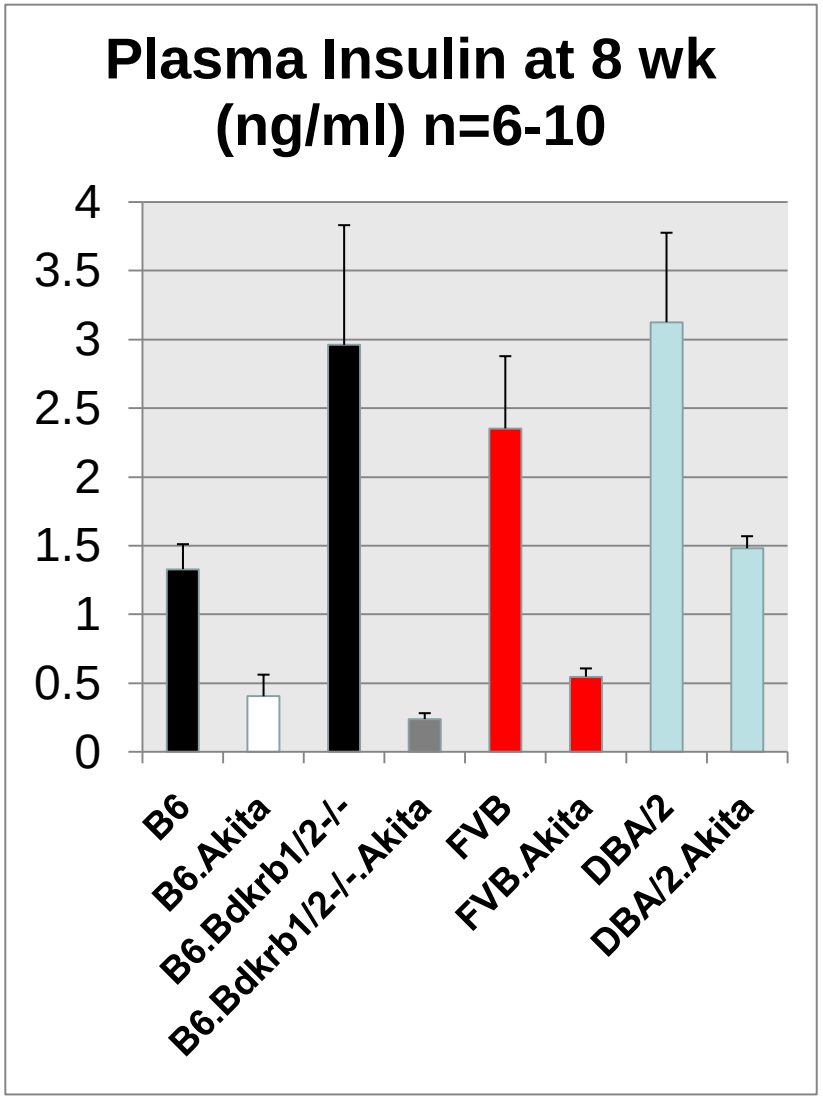
Glucose and Insulin in Males





NIH Rat & Mouse/Auto 11F 5LA6

GUARATEED ANALYSIS

Crude protein not less than	18.0
Crude fat not less than	11.0
Crude fiber not more than	5.0

INGREDIENTS

Ground wheat, wheat middlings, ground oats, dehulled soybean meal, fish meal, ground corn, soybean oil, corn gluten meal, dehydrated alfalfa meal, dicalcium phosphate, brewers dried yeast, menadione dimethylpyrimidinol bisulfite (source of vitamin K), calcium carbonate, salt, choline chloride, magnesium oxide, thiamin mononitrate, pyridoxine hydrochloride, cholecalciferol (source of vitamin D-3), vitamin A acetate, calcium pantothenate, biotin, ferrous sulfate, manganous oxide, cyanocobalamin (source of vitamin B-12), folic acid, riboflavin, nicotinic acid, dl-alpha tocopheryl acetate, calcium iodate, zinc oxide, ferrous carbonate, copper sulfate, zinc sulfate, cobalt carbonate.

FEEDING DIRECTIONS

Feed ad libitum. Plenty of fresh, clean water should be available at all times.

CHEMICAL COMPOSITION[1]

Nutrients[2]

Protein, %	**19.7**
Arginine, %	1.14
Cystine, %	0.27
Glycine, %	1.00
Histidine, %	0.45
Isoleucine, %	1.06
Leucine, %	1.61
Lysine, %	1.04
Methionine, %	0.39
Phenylalanine, %	0.92
Tyrosine, %	0.56
Threonine, %	0.74
Tryptophan, %	0.25
Valine, %	1.01
Serine, %	0.99
Aspartic Acid, %	1.91
Glutamic Acid, %	4.52
Alanine, %	1.11
Proline, %	1.50
Taurine, %	0.00
Fat (ether extract), %	**12.2**
Fat (acid hydrolysis), %	**13.1**
Cholesterol, ppm	0.0
Linoleic Acid, %	5.88
Linolenic Acid, %	0.84
Arachidonic Acid, %	0.02
Omega-3 Fatty Acids, %	1.06
Total Saturated Fatty Acids, %	2.14
Total Monounsaturated Fatty Acids, %	2.63
Fiber (crude), %	**4.0**
Neutral Detergent Fiber[3], %	14.5
Acid Detergent Fiber[4], %	5.2
Sulfur, %	0.24
Sodium, %	0.28
Chlorine, %	0.47
Fluorine, ppm	6.8
Iron, ppm	258
Zinc, ppm	88
Manganese, ppm	147
Copper, ppm	11
Cobalt, ppm	1.09
Iodine, ppm	2.08
Chromium, ppm	0.54
Selenium, ppm	0.31

Vitamins

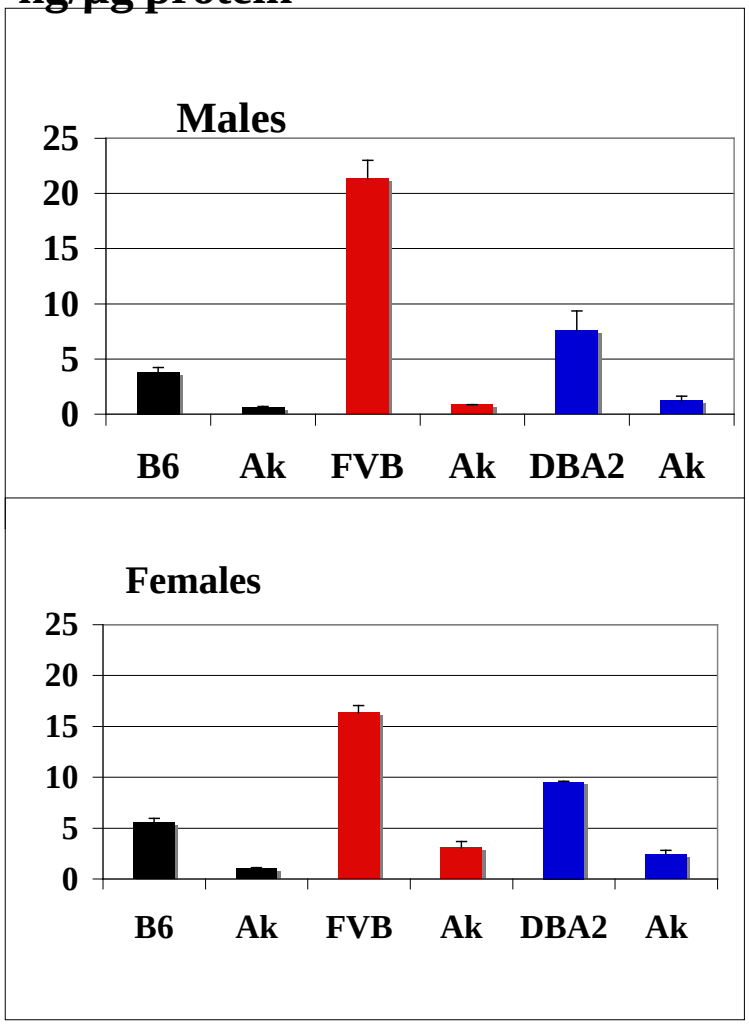
Carotene, ppm	1.5
Vitamin K (as menadione), ppm	22.5
Thiamin Hydrochloride, ppm	88.0
Riboflavin, ppm	8.1
Niacin, ppm	88
Pantothenic Acid, ppm	41
Choline Chloride, ppm	2,046
Folic Acid, ppm	2.1
Pyridoxine, ppm	9.7
Biotin, ppm	0.3
Vitamin B-12, mcg/kg	70
Vitamin A, IU/g	24
Vitamin D-3 (added), IU/g	4.3
Vitamin E, IU/kg	27
Ascorbic Acid, ppm	0

Calories provided by:

Protein, %	20.805
Fat (ether extract), %	28.990

Insulin, 5 Weeks n=3

Pancreatic Insulin Content, ng/µg protein



Plasma Insulin, ng/ml