Manuscript Title: **Phytochemical compound PB125 attenuates skeletal muscle mitochondrial dysfunction and impaired proteostasis in a preclinical model of musculoskeletal decline**

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Animal model used, if applicable Guinea pig – Dunkin Hartley Guinea pig

Underlying hypothesis This study tests the hypothesis that 10 months of PB125 supplementation would attenuate the age/disease related declines in skeletal muscle of Hartley guinea pigs, particularly the declines in mitochondrial respiration and protein synthesis. We were additionally interested in testing the hypothesis that attenuation of the declines in mitochondrial respiration and protein synthesis would yield to maintained mobility in Hartley guinea pigs as their musculoskeletal dysfunction progressed.

Definitions of 'n':

Q1 The number of guinea pigs. Weekly measurements of weight were used to generate growth curves from all guinea pigs.
Q2 Refers to averaged technical duplicates acquired from biological sample (permeabilized soleus myofibers). Samples were excluded if CCF was beyond cutoff. Outlier test used to remove any outliers. Refer to methods for details of exclusion.
Q3 Refers to averaged technical duplicates acquired from biological sample (permeabilized soleus myofibers). Samples were excluded if CCF was beyond cutoff. Outlier test used to remove any outliers. Refer to methods for details of exclusion.
Q4 Refers to averaged technical duplicates acquired from biological sample (permeabilized soleus myofibers). Samples were excluded if CCF was beyond cutoff. Outlier test used to remove any outliers. Refer to methods for details of exclusion.
Q5 Refers to averaged technical duplicates acquired from biological sample (permeabilized soleus myofibers). Samples were excluded if CCF was beyond cutoff. Outlier test used to remove any outliers. Refer to methods for details of exclusion.
Q6 Refers to averaged technical duplicates acquired from biological sample (permeabilized soleus myofibers). Samples were excluded if CCF was beyond cutoff. Outlier test used to remove any outliers. Refer to methods for details of exclusion.
Q7 Fractionated skeletal muscle tissue of guinea pigs.
Q8 Fractionated skeletal muscle tissue of guinea pigs.
Q9 Fractionated skeletal muscle tissue of guinea pigs.
Q10 Homogenized skeletal muscle tissue of subset of guinea pigs
Q11 Homogenized skeletal muscle tissue of subset of guinea pigs
Q12 The number of guinea pigs that were part of the long term study (excludes guinea pigs that died prematurely.

Experimental Question #	Finding Conclusion	Experimental location/variable	Summary Statistics (Mean, SD, n)							Units	Statistical test	Any other variable	Figure/table in which data are presented	Comments
	The rate of growth was not different between treated and untreated male guinea pigs.	Rate of growth (K)	K values	CON:0.08706 PB125:0.08314	95% CI	CON: PB125:	0.07213 – 0.06939 – 0.09784	DFd: 1120 28 animals	(reflects data points used to generate curve) p value 0.7151	k	CON vs PB125		Fig 1F	Growth rates (k) were calculated by weekly measurements of mass for each guinea pig over the 10 month period (reflected by
	The rate of growth was not different between treated and untreated female guinea pigs.	Rate of growth (K)	K values	CON:0.07667 PB125: 0.07244	95% CI	CON: PB125:	0.06170 – 0.09263 0.05027 – 0.09687	DFd: 1049 28 animals	(reflects data points used to generate curve) 0.7593	k	CON vs PB125		Fig 1G	Growth rates (k) were calculated by weekly measurements of mass for each guinea pig over the 10 month period (reflected by DFd)

1. Is growth or size different between treated and untreated guinea pigs?	Finding Conclusion	Experimental location/variable	Summary Statistics (Mean, SD, n)												Units	Statistical test	Any other variable	Figure/table in which data are presented	Comments
				Male CON			Male PB125			Female CON			Female PB125				Three Way ANOVA			
	Body mass was not different between treated and untreated guinea pigs	Body mass		Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	grams	Age: <0.0001, Sex: <0.0001 Treatment: 0.7693		Fig 1H	
			5mo	933	71.9355772	12	929.454545	71.3755751	11	768	62.3409977	11	765.833333	75.9591198	12					
			15mo	1174.83333	151.659207	12	1205.8	93.5043671	10	1002.6	98.6894118	10	1000.8	84.9441647	10					
	Relative soleus mass was not different between treated and untreated guinea pigs.	Soleus mass divided by body mass		Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	relative mass (muscle mass [mg] / body mass [g])	Age: 0.1148, Sex: 0.4181, Treatment: 0.1611		Fig 1I	
			5mo	0.22714667	0.03191312	12	0.22068909	0.03623283	11	0.23381091	0.04446527	11	0.2355475	0.04152482	12					
			15mo	0.22258333	0.04945789	12	0.20233333	0.04960847	9	0.23033333	0.05332917	9	0.2031	0.03058122	10					
	Relative gastrocnemius mass was not different between treated and untreated guinea pigs	Gastrocnemius mass divided by body mass		Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	relative mass (muscle mass [mg] / body mass [g])	Age: 0.0050, Sex: 0.558, Treatment: 0.5781		Fig 1J	
			5mo	1.9676675	0.1261685	12	1.99459	0.2061759	11	2.01765364	0.1972424	11	2.031985	0.17281013	12					
			15mo	1.85941667	0.20458404	12	1.90322222	0.23644808	9	1.88288889	0.17785629	9	1.89	0.19404581	10					

Experimental Question #	Finding Conclusion	Experimental location/variable		Summary Statistics (Mean, SD, n)												Units	Statistical test	Any other variable	Figure/table in which data are presented	Comments
				Male CON			Male PB125			Female CON			Female PB125				Three Way ANOVA			
2. Are there Disease/Age-, Sex-, and Treatment related effects on skeletal muscle mitochondrial respiration?	There are significant main effects of disease/age and sex on State 3 mitochondrial respiration, but not treatment.	Permeabilized myofibers of soleus		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	oxygen consumption (pmol O2/(mg*s))	Age: 0.0012, Sex: 0.0057, Treatment: 0.0979		Fig 3A	
			5mo	99.5276958	18.66274	12	97.5147423	23.4106458	13	88.5799643	14.0457714	14	98.7043107	16.6658579	14					
			15mo	85.8028571	24.9938572	14	97.4923077	24.7245415	13	70.4323077	16.9556216	13	77.3766667	21.585462	12					
	There are significant main effects of disease/age, sex, and treatment on ETS capacity (Complex I - IV).	Permeabilized myofibers of soleus		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	oxygen consumption (pmol O2/(mg*s))	Age: 0.0038, Sex: 0.0018, Treatment: 0.0369		Fig 3B	
			5mo	107.410796	17.3659599	13	107.471711	23.9735865	14	95.6786929	12.1086217	14	108.909382	16.7000258	14					
			15mo	95.3414286	28.2551403	14	111.15	22.079589	13	80.2938462	20.1534776	13	85.1191667	20.7205377	12					
	There are significant main effects of disease/age and sex on ETS capacity (Complex II - IV), but not treatment.	Permeabilized myofibers of soleus		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	oxygen consumption (pmol O2/(mg*s))	Age: <0.0001, Sex: 0.0015, Treatment: 0.3686		Fig 3C	
			5mo	72.3387346	13.1018151	13	71.8934643	12.2785257	14	64.8530143	10.183499	14	68.6162786	9.4492954	14					
			15mo	56.0421429	10.7295983	14	61.02	13.0105521	13	49.1330769	12.814388	13	49.035	11.9533864	12					
	There is a significant main effect of disease/age on RCR, but not sex or treatment.	Permeabilized myofibers of soleus		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	Ratio	Age: 0.0117, Sex: 0.5763, Treatment: 0.4027		Fig 3D	
			5mo	13.7375241	2.92464774	11	12.4175548	5.91692153	12	11.6563386	3.50597489	13	14.1583086	4.77056906	13					
			15mo	11.164983	5.84144599	14	11.0785241	4.47831446	13	9.34990257	2.24577659	13	11.2413266	4.35138311	12					
				Male CON			Male PB125			Female CON			Female PB125				Three Way ANOVA			
3. Are there Disease/Age-, Sex-, and Treatment related effects on fatty acid supported skeletal muscle mitochondrial respiration?	There are no effects of disease/age and sex on State 3 fatty acid supported mitochondrial respiration with 0.5 mM ADP present.	Permeabilized myofibers of soleus		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	oxygen consumption (pmol O2/(mg*s))	Age: 0.8125, Sex: 0.5343, Treatment: 0.8930		Fig 3E	
			5mo	42.0946167	5.53852908	6	36.3777	10.5266758	7	37.3601167	2.43270933	6	39.6061389	5.43781586	9					
			15mo	41.1203136	7.47993877	11	39.1209091	8.20109926	11	35.255	6.91944493	12	41.75	10.6846307	7					
	There is a significant effect of treatment on State 3 fatty acid supported mitochondrial respiration with 1.0 mM ADP present, but no effects of either Sex or Treatment.	Permeabilized myofibers of soleus		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	oxygen consumption (pmol O2/(mg*s))	Age: 0.8789, Sex: 0.1205, Treatment: 0.0349		Fig 3F	
			5mo	44.0325636	8.13458713	11	50.6090962	13.2921892	13	42.0969409	3.45188836	11	45.1631321	6.86170343	14					
			15mo	46.9315636	9.22131165	11	45.7272727	5.37350926	11	40.0916667	8.23578537	12	47.9957143	13.228866	7					
	There was no significant effect of sex, disease/age, or treatment on State 3 fatty acid supported mitochondrial respiration with 6.0 mM ADP present.	Permeabilized myofibers of soleus		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	oxygen consumption (pmol O2/(mg*s))	Age: 0.1584, Sex: 0.0695, Treatment: 0.0773		Fig 3G	
			5mo	62.0050864	7.80694986	11	63.8228385	18.9146688	13	53.6398455	5.84251776	11	62.0279929	10.281103	14					
			15mo	58.5307682	12.2325652	11	59.38	10.6120177	11	50.0083333	13.3945415	12	58.2357143	16.7850676	7					

Experimental Question #	Finding Conclusion	Experimental location/variable		Summary Statistics (Mean, SD, n)												Units	Statistical test	Any other variable	Figure/table in which data are presented	Comments
				Male CON			Male PB125			Female CON			Female PB125				Three Way ANOVA			
4. Are there Disease/Age-, Sex-, and Treatment related effects on ROS emission in skeletal muscle mitochondria?	There are no effects of disease/age, treatment, or sex on maximum LEAK.	Permeabilized myofibers of soleus		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	ROS Emission (pmol/(mg*s))	Age: 0.1234, Sex: 0.1602 Treatment: 0.3179		Fig 3H	
			5mo	0.42805336	0.08043792	11	0.43372192	0.13929264	12	0.45623588	0.18622498	12	0.38816307	0.10110131	14					
			15mo	0.40372545	0.13585671	11	0.39790111	0.07993834	9	0.3620104	0.09612577	10	0.39574357	0.17953402	7					
	There are no effects of disease/age, treatment, or sex on ROS emission during State 3 respiration supported by subsaturating amounts of ADP.	Permeabilized myofibers of soleus		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	ROS Emission (pmol/(mg*s))	Age: 0.2551, Sex: 0.9241, Treatment: 0.9641		Fig 3G	
			5mo	0.21674	0.07113392	6	0.22606338	0.05194098	8	0.23345815	0.08701785	6	0.23281856	0.05402247	9					
			15mo	0.19293364	0.06461414	11	0.20461111	0.03942281	9	0.2092293	0.04340844	10	0.21227786	0.03719251	7					
				Male CON			Male PB125			Female CON			Female PB125				Three Way ANOVA			
5. Are there Disease/Age-, Sex-, and Treatment related effects on ADP Kinetics in skeletal muscle mitochondria?	There are significant effects of Disease/Age, Sex, and Treatment on ADP Vmax. In addition, PB125 treated 5 mo females have greater respiration than 5 mo CON.	Permeabilized myofibers of soleus		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	oxygen consumption (pmol O2/(mg*s))	Age: 0.0495, Sex: 0.0010, Treatment: 0.0261, Age x Sex x Treatment: 0.0210		Fig 3I	
			5mo	50.2081818	6.54644914	11	50.3407692	14.1048741	13	39.3530769	8.53556519	13	52.3653846	10.5011163	13					
			15mo	54.12	11.8800891	13	61.6061538	11.855594	13	47.3830769	13.3609271	13	46.8591667	9.71323133	12					
	There is a significant effect of treatment on ADP Km (sensitivity), but no effect of sex or age	Permeabilized myofibers of soleus		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	concentration of ADP	Age: 0.4023, Sex: 0.1311, Treatment: 0.0069		Fig 3J	
			5mo	0.488775	0.21902066	12	0.83565833	0.57318332	12	0.43314167	0.16610897	12	0.62755455	0.16896954	11					
			15mo	0.56958571	0.33010164	14	0.79308	0.19993335	10	0.64803077	0.33967186	13	0.58931667	0.24076722	12					
	PB125 treatment augments increase in ADP Vmax	permeabilized myofibers of soleus		**Mean**	**SD**	**n**													Fig 4A	
			5mo CON	44.33	9.337	24	oxygen consumption (pmol O2/(mg*s))	One-Way ANOVA Dunnett's multiple comparisons test		**p value**										
			15mo CON	50.75	12.85	26		5mo CON vs. 15mo CON		0.1085										
			15mo PB125	54.53	13.04	25		5mo CON vs. 15mo PB125		0.0072										
	PB125 treatment augments increase in ADP Vmax in males only	permeabilized myofibers of soleus			5mo CON			15mo CON			15mo PB125					**Two-Way ANOVA Dunnett's multiple comparisons test**				Fig 4B	
				Mean	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	oxygen consumption (pmol O2/(mg*s))	5mo CON vs 15mo CON	0.5692	5mo CON vs 15mo PB125	0.0209			
			Male	50.2081818	6.54644914	11	54.12	11.8800891	13	61.6061538	11.855594	13								
			Female	39.3530769	8.53556519	13	47.3830769	13.3609271	13	46.8591667	9.71323133	12		5mo CON vs 15mo CON	0.1067	5mo CON vs 15mo PB125	0.1479			
	PB125 attenuates the age-related decline in State	permeabilized myofibers of soleus		**Mean**	**SD**	**n**													Fig 4C	
			5mo CON	93.63	16.94	26	oxygen consumption (pmol	One-Way ANOVA Dunnett's multiple comparisons test		**p value**										
			15mo CON	78.4	22.5	27		5mo CON vs. 15mo CON		0.0238										

	3 respiration.		**15mo PB125**	87.84	24.9	25	O2/(mg*s))	5mo CON vs. 15mo PB125			0.5358														
	PB125 attenuates the age-related decline in State 3 respiration in females only	permeabilized myofibers of soleus		5mo CON			15mo CON			15mo PB125			Two-Way ANOVA Dunnett's multiple comparisons test											Fig 4D
				Mean	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**													
			Male	99.5276958	18.66274	12	85.8028571	24.9938572	14	97.4923077	24.7245415	13	oxygen consumption (pmol O2/(mg*s))	5mo CON vs 15mo CON	0.1633	5mo CON vs 15mo PB125	0.9554							
			Female	88.5799643	14.0457714	14	70.4323077	16.9556216	13	77.3766667	21.585462	12		5mo CON vs 15mo CON	0.0463	5mo CON vs 15mo PB125	0.2898							
	PB125 does not attenuate the disease/age related decline in ETS capacity (because there was not a significant decline in ETS capacity in this	permeabilized myofibers of soleus		**Mean**	**SD**	**n**																			
			5mo CON	101.3	15.75	27		One-Way ANOVA Dunnett's multiple comparisons test			**p value**														
			15mo CON	88.1	25.4	27	oxygen consumption (pmol O2/(mg*s))	5mo CON vs. 15mo CON			0.0605														
			15mo PB125	98.66	24.83	25		5mo CON vs. 15mo PB125			0.8752														
6. Does PB125 treatment modulate disease/age-related changes in mitochondrial respiration? Are there any sex specific effects?	PB125 does not sex speciifcally attenuate the age-related decline in ETS capacity	permeabilized myofibers of soleus		5mo CON			15mo CON			15mo PB125			Two-Way ANOVA Dunnett's multiple comparisons test											Fig 4F
				Mean	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**													
			Male	107.410796	17.3659599	13	95.3414286	28.2551403	14	111.308462	22.192122	13	oxygen consumption (pmol O2/(mg*s))	5mo CON vs 15mo CON	0.2314	5mo CON vs 15mo PB125	0.8467							
			Female	95.6786929	12.1086217	14	80.2938462	20.1534776	13	85.1191667	20.7205377	12		5mo CON vs 15mo CON	0.1052	5mo CON vs 15mo PB125	0.3354							
	PB125 does not attenuate the disease/age related decline in ETS capacity (CII- CIV)	permeabilized myofibers of soleus		**Mean**	**SD**	**n**																			
			5mo CON	68.46	12.07	27		One-Way ANOVA Dunnett's multiple comparisons test			**p value**														
			15mo CON	52.72	12.07	27	oxygen consumption (pmol O2/(mg*s))	5mo CON vs. 15mo CON			<0.0001														
			15mo PB125	55.27	13.69	25		5mo CON vs. 15mo PB125			0.0006														
	PB125 does not sex speciifcally attenuate the age-related decline in ETS capacity (CII - CIV)	permeabilized myofibers of soleus		5mo CON			15mo CON			15mo PB125			Two-Way ANOVA Dunnett's multiple comparisons test											Fig 4H
				Mean	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**													
			Male	72.3387346	13.1018151	13	56.0421429	10.7295983	14	61.02	13.0105521	13	oxygen consumption (pmol O2/(mg*s))	5mo CON vs 15mo CON	0.0014	5mo CON vs 15mo PB125	0.0345							
			Female	64.8530143	10.183499	14	49.1330769	12.814388	13	49.035	11.9533864	12		5mo CON vs 15mo CON	0.0021	5mo CON vs 15mo PB125	0.0025							
	PB125 attenuates the disease/age related decline in RCR	permeabilized myofibers of soleus		**Mean**	**SD**	**n**																			
			5mo CON	12.61	3.555	24		One-Way ANOVA Dunnett's multiple comparisons test			**p value**														
			15mo CON	9.708	3.391	26	ratio	5mo CON vs. 15mo CON			<0.0001														
			15mo PB125	11.16	4.326	25		5mo CON vs. 15mo PB125			0.0006														
	PB125 attenuates the disease/age related decline in RCR in males only (no age-related decline in females)	permeabilized myofibers of soleus		5mo CON			15mo CON			15mo PB125			Two-Way ANOVA Dunnett's multiple comparisons test											Fig 4J
				Mean	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**													
			Male	13.7375241	2.92464774	11	10.0654575	4.31627083	13	11.0785241	4.47831446	13	ratio	5mo CON vs 15mo CON	0.0356	5mo CON vs 15mo PB125	0.01516							
			Female	11.6563386	3.50597489	13	9.34990257	2.24577659	13	11.2413266	4.35138311	12		5mo CON vs 15mo CON	0.2093	5mo CON vs 15mo PB125	0.9458							

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	There is a significant effect of disease/age on myofibrillar protein synthesis in the soleus	Myofibrillar subfraction of Soleus muscle		Male CON			Male PB125			Female CON			Female PB125				Three Way ANOVA			
				Mean	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**					
			5mo	2.22549538	0.1338218	13	2.27587718	0.16137748	13	2.34915667	0.26437344	14	2.22320641	0.18728575	13	fractional synthesis rate (%/day)	Age: 0.0015, Sex: 0.5587, Treatment: 0.4982		Fig 5A	
			15mo	2.09324976	0.18108292	14	2.20301389	0.18551335	12	2.12306806	0.16032488	12	2.18618639	0.12621223	12					

7. Are there Disease/Age-, Sex-, and Treatment related effects on skeletal muscle protein synthesis?	There is a significant effect of disease/age on mitochondrial protein synthesis in the soleus	Mitochondrial subfraction of Soleus muscle		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	fractional synthesis rate (%/day)	Age: 0.0029, Sex: 0.7811, Treatment: 0.3887		Fig 5B
			5mo	1.7374618	0.21034142	13	1.72790714	0.16061741	14	1.7425441	0.23958803	13	1.80200905	0.30720095	14				
			15mo	1.59909881	0.19148393	14	1.69472095	0.19286796	12	1.57687806	0.18026636	12	1.5874975	0.30541418	12				
	There is a significant effect of disease/age on cytosolic protein synthesis in the soleus	Cytosolic subfraction of Soleus muscle		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	fractional synthesis rate (%/day)	Age: <0.0001, Sex: 0.1457, Treatment: 0.2072		Fig 5C
			5mo	2.31875462	0.1373844	13	2.39275643	0.20134459	14	2.42844222	0.1795843	12	2.43478262	0.35830419	14				
			15mo	2.15263571	0.14790641	14	2.22183778	0.16792303	12	2.2020925	0.19777053	12	2.25817139	0.12983812	12				
	There is a significant effect of disease/age on Collagen protein synthesis in the soleus	Collagen subfraction of Soleus muscle		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	fractional synthesis rate (%/day)	Age: <0.0001, Sex: 0.4539, Treatment: 0.8824		Fig 5D
			5mo	0.91436128	0.23069098	13	0.97412077	0.40340964	13	0.95704513	0.43079109	13	0.81466718	0.20758125	13				
			15mo	0.6040056	0.31295196	14	0.59454028	0.28731824	12	0.49936389	0.21940987	12	0.6284425	0.33627904	12				
	There is a significant effect of disease/age on myofibrillar protein synthesis in the gastrocnemius	Myofibrillar subfraction of gastrocnemius muscle		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	fractional synthesis rate (%/day)	Age: <0.0001, Sex: 0.9541, Treatment: 0.4042		Fig 5E
			5mo	1.93359333	0.14684657	13	1.99464857	0.15391676	14	1.96200154	0.13141966	13	1.99989103	0.3017671	13				
			15mo	1.71260976	0.13716658	14	1.73493436	0.15787186	12	1.70658614	0.18465984	12	1.6994478	0.07726639	12				
	There is a significant effect of disease/age on mitochondrial protein synthesis in the gastrocnemius	Mitochondrial subfraction of gastrocnemius muscle		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	fractional synthesis rate (%/day)	Age: <0.0001, Sex: 0.9226, Treatment: 0.9433		Fig 5F
			5mo	1.88033974	0.13536286	13	1.89296846	0.18749823	13	1.83008461	0.14552234	13	1.89414026	0.31656624	13				
			15mo	1.69435933	0.17251916	14	1.6289415	0.12684399	12	1.68996803	0.16671628	12	1.66856568	0.06898468	12				
	There is a significant effect of disease/age on cytosolic protein synthesis in the gastrocnemius	Cytosolic subfraction of gastrocnemius muscle		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	fractional synthesis rate (%/day)	Age: <0.0001, Sex: 0.1948, Treatment: 0.4439		Fig 5G
			5mo	2.00498769	0.13776247	13	2.08004821	0.14146105	13	2.14378051	0.22920456	13	2.15360048	0.29833302	14				
			15mo	1.81458095	0.11138432	14	1.82554111	0.19333809	12	1.80076306	0.16847819	12	1.8164975	0.07900233	12				
	There is a significant effect of disease/age on collagen protein synthesis in the gastrocnemius	Collagen subfraction of gastrocnemius muscle		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	fractional synthesis rate (%/day)	Age: <0.0001, Sex: 0.2064, Treatment: 0.5310		Fig 5H
			5mo	1.46383154	0.22666781	13	1.52458881	0.41531949	14	1.52812897	0.23259949	13	1.56839128	0.41492193	13				
			15mo	1.14150524	0.2450633	14	1.06753111	0.32596463	12	1.29843417	0.17711874	12	1.11631778	0.35655402	12				

Experimental Question #	**Finding Conclusion**	**Experimental location/variable**		**Summary Statistics (Mean, SD, n)**									**Units**	**Statistical test**	**Any other variable**	**Figure/table in which data are presented**	**Comments**
	PB125 treatment attenuates the age related decline in FSR in the myofibrillar fraction of the soleus	Myofibrillar subfraction of Soleus muscle		**Mean**	**SD**	**n**							fractional synthesis rate (%/day)	One-Way ANOVA Dunnett's multiple comparisons test	**p value**	Fig 6A	
			5mo CON	2.262	0.1673	26											
			15mo CON	2.107	0.1691	26								5mo CON vs. 15mo CON	0.0021		
			15mo PB125	2.195	0.1554	24								5mo CON vs. 15mo PB125	0.2566		
	PB125 treatment attenuates the age related decline in FSR in the myofibrillar fraction of the soleus in females only	Myofibrillar subfraction of Soleus muscle		5mo CON			15mo CON			15mo PB125			fractional synthesis rate (%/day)	**Two-Way ANOVA Dunnett's multiple comparisons test**		Fig 6B	
				Mean	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**		5mo CON vs 15mo CON	0.0769		
			Male	2.22549538	0.1338218	13	2.09324976	0.18108292	14	2.20301389	0.18551335	12		5mo CON vs 15mo PB125	0.9201		
			Female	2.29895667	0.19364181	13	2.12306806	0.16032488	12	2.18618639	0.12621223	12		5mo CON vs 15mo CON	0.019		
														5mo CON vs 15mo PB125	0.1661		
	PB125 treatment attenuates the age related decline in FSR in the mitochondrial fraction of the soleus	Myofibrillar subfraction of Soleus muscle		**Mean**	**SD**	**n**							fractional synthesis rate (%/day)	One-Way ANOVA Dunnett's multiple comparisons test	**p value**	Fig 6C	
			5mo CON	1.74	0.2209	26											
			15mo CON	1.589	0.183	26								5mo CON vs. 15mo CON	0.0299		
			15mo PB125	1.641	0.2557	24								5mo CON vs. 15mo PB125	0.2051		

Experimental Question #	Finding Conclusion	Experimental location/variable		Summary Statistics (Mean, SD, n)									Units	Statistical test	Any other variable	Figure/table in which data are presented	Comments
8. Does PB125 treatment modulate disease/age-related changes in protein synthesis in the soleus? Are there any sex specific effects?	PB125 treatment attenuates the age related decline in FSR in the mitochondrial fraction of the	Mitochondrial subfraction of Soleus muscle		5mo CON			15mo CON			15mo PB125			fractional synthesis rate (%/day)	Two-Way ANOVA Dunnett's multiple comparisons test		Fig 6D	
				Mean	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**		5mo CON vs 15mo CON	0.1948		
			Male	1.7374618	0.21034142	13	1.59909881	0.19148393	14	1.69472095	0.19286796	12		5mo CON vs 15mo PB125	0.8481		
			Female	1.7425441	0.23958803	13	1.57687806	0.18026636	12	1.5874975	0.30541418	12		15mo CON vs 15mo PB125	0.1221		
	PB125 treatment does not attenuate the age related decline in FSR in the cytosolic fraction of the soleus when pooling male and female guinea pigs	Cytosolic subfraction of Soleus muscle		**Mean**	**SD**	**n**										Fig 6E	
			5mo CON	2.371	0.1654	25							fractional synthesis rate (%/day)	One-Way ANOVA Dunnett's multiple comparisons test	**p value**		
			15mo CON	2.158	0.1479	25								5mo CON vs. 15mo CON	<0.0001		
			15mo PB125	2.24	0.148	24								5mo CON vs. 15mo PB125	0.0075		
	PB125 treatment attenuates the age related decline in FSR in the cytosolic fraction of the soleus in males only	Cytosolic subfraction of Soleus muscle		5mo CON			15mo CON			15mo PB125			fractional synthesis rate (%/day)	Two-Way ANOVA Dunnett's multiple comparisons test		Fig 6F	
				Mean	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**		5mo CON vs 15mo CON	0.0123		
			Male	2.31875462	0.1373844	13	2.15263571	0.14790641	14	2.22183778	0.16792303	12		5mo CON vs 15mo PB125	0.2065		
			Female	2.42844222	0.1795843	12	2.16412727	0.15491472	11	2.25817139	0.12983812	12		15mo CON vs 15mo PB125	0.0002		
	PB125 treatment does not attenuate the age related decline in FSR in the collagen fraction of the soleus	Collagen subfraction of Soleus muscle		**Mean**	**SD**	**n**										Fig 6G	
			5mo CON	0.9357	0.3393	26							fractional synthesis rate (%/day)	One-Way ANOVA Dunnett's multiple comparisons test	**p value**		
			15mo CON	0.5557	0.2738	26								5mo CON vs. 15mo CON	<0.0001		
			15mo PB125	0.6115	0.3064	24								5mo CON vs. 15mo PB125	0.0008		
	PB125 treatment does not attenuate the age related decline in FSR in the collagen fraction of the soleus in either males or females	Collagen subfraction of Soleus muscle		5mo CON			15mo CON			15mo PB125			fractional synthesis rate (%/day)	Two-Way ANOVA Dunnett's multiple comparisons test		Fig 6H	
				Mean	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**		5mo CON vs 15mo CON	0.0225		
			Male	0.91436128	0.23069098	13	0.6040056	0.31295196	14	0.59454028	0.28731824	12		5mo CON vs 15mo PB125	0.0238		
			Female	0.95704513	0.43079109	13	0.49936389	0.21940987	12	0.6284425	0.33627904	12		15mo CON vs 15mo PB125	0.001		
	PB125 treatment does not attenuate the age related decline in FSR in the myofibrillar fraction of the	Myofibrillar subfraction of gastrocnemius muscle		**Mean**	**SD**	**n**										Fig 6I	
			5mo CON	1.948	0.1373	26							fractional synthesis rate (%/day)	One-Way ANOVA Dunnett's multiple comparisons test	**p value**		
			15mo CON	1.71	0.1575	26								5mo CON vs. 15mo CON	<0.0001		
			15mo PB125	1.717	0.1229	24								5mo CON vs. 15mo PB125	<0.0001		
	PB125 treatment does not attenuate the age related decline in FSR in the myofibrillar fraction of the gastrocnemius in either male or females	Myofibrillar subfraction of gastrocnemius muscle		5mo CON			15mo CON			15mo PB125			fractional synthesis rate (%/day)	Two-Way ANOVA Dunnett's multiple comparisons test		Fig 6J	
				Mean	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**		5mo CON vs 15mo CON	0.0003		
			Male	1.93359333	0.14684657	13	1.71260976	0.13716658	14	1.73493436	0.15787186	12		5mo CON vs 15mo PB125	0.0017		
			Female	1.96200154	0.13141966	13	1.70658614	0.18465984	12	1.6994478	0.07726639	12		15mo CON vs 15mo PB125	<0.0001		
	PB125 treatment attenuates the age related decline in FSR in the mitochondrial fraction of the	Myofibrillar subfraction of gastrocnemius muscle		**Mean**	**SD**	**n**										Fig 6K	
			5mo CON	1.855	0.1401	26							fractional synthesis rate (%/day)	One-Way ANOVA Dunnett's multiple comparisons test	**p value**		
			15mo CON	1.692	0.1665	26								5mo CON vs. 15mo CON	0.0001		
			15mo PB125	1.649	0.1019	24								5mo CON vs. 15mo PB125	<0.0001		
	PB125 treatment			5mo CON			15mo CON			15mo PB125							

Experimental Question #	Finding Conclusion	Experimental location/variable		Summary Statistics (Mean, SD, n)												Units	Statistical test	Any other variable	Figure/table in which data are presented	Comments
9. Does PB125 treatment modulate disease/age-related changes in protein synthesis in the gastrocnemius? Are there any sex specific effects?	attenuates the age related decline in FSR in the mitochondrial fraction of the soleus is not	Mitochondrial subfraction of gastrocnemius muscle		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**					**Two-Way ANOVA Dunnett's multiple comparisons test**				
			Male	1.88033974	0.13536286	13	1.69435933	0.17251916	14	1.6289415	0.12684399	12	fractional synthesis rate (%/day)	5mo CON vs 15mo CON	0.0021	5mo CON vs 15mo PB125	<0.0001		Fig 6L
			Female	1.83008461	0.14552234	13	1.68996803	0.16671628	12	1.66856568	0.06898468	12		5mo CON vs 15mo CON	0.0294	15mo PB125	0.0108		
	PB125 treatment does not attenuate the age related decline in FSR in the cytosolic fraction of the soleus when pooling male and female guinea pigs together	Cytosolic subfraction of gastrocnemius muscle		**Mean**	**SD**	**n**														
			5mo CON	2.074	0.1983	26							fractional synthesis rate (%/day)	One-Way ANOVA Dunnett's multiple comparisons test	**p value**					
			15mo CON	1.808	0.1378	26								5mo CON vs. 15mo CON	<0.0001					Fig 6M
			15mo PB125	1.821	0.1445	24								5mo CON vs. 15mo PB125	<0.0001					
	PB125 treatment attenuates the age related decline in FSR in the cytosolic fraction of the soleus in males only	Cytosolic subfraction of gastrocnemius muscle		5mo CON			15mo CON			15mo PB125										
				Mean	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**					**Two-Way ANOVA Dunnett's multiple comparisons test**				
			Male	2.00498769	0.13776247	13	1.81458095	0.11138432	14	1.82554111	0.19333809	12	fractional synthesis rate (%/day)	5mo CON vs 15mo CON	0.0058	5mo CON vs 15mo PB125	0.0131		Fig 6N
			Female	2.14378051	0.22920456	13	1.80076306	0.16847819	12	1.8164975	0.07900233	12		5mo CON vs 15mo CON	<0.0001	15mo PB125	<0.0001		
	PB125 treatment does not attenuate the age related decline in FSR in the collagen fraction of the soleus	Collagen subfraction of gastrocnemius muscle		**Mean**	**SD**	**n**														
			5mo CON	1.496	0.2274	26							fractional synthesis rate (%/day)	One-Way ANOVA Dunnett's multiple comparisons test	**p value**					
			15mo CON	1.214	0.2267	26								5mo CON vs. 15mo CON	0.0005					Fig 6O
			15mo PB125	1.092	0.335	24								5mo CON vs. 15mo PB125	<0.0001					
	PB125 treatment does not attenuate the age related decline in FSR in the collagen fraction of the soleus in either males or females	Collagen subfraction of gastrocnemius muscle		5mo CON			15mo CON			15mo PB125										
				Mean	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**					**Two-Way ANOVA Dunnett's multiple comparisons test**				
			Male	1.46383154	0.22666781	13	1.14150524	0.2450633	14	1.06753111	0.32596463	12	fractional synthesis rate (%/day)	5mo CON vs 15mo CON	0.0047	15mo PB125	0.0008		Fig 6P
			Female	1.52812897	0.23259949	13	1.29843417	0.17711874	12	1.11631778	0.35655402	12		5mo CON vs 15mo CON	0.0636	5mo CON vs 15mo PB125	0.0005		
10. Are there Disease/Age-, Sex-, and Treatment related effects on Nrf2 or HO-1 content in the gastrocnemius?	There are significant effects of Disease/Age and Treatment, but not Sex, on Nrf2 content in the gastrocnemius. There is a significant interaction between Disease/Age and Treatment	Homogenized gastrocnemius		Male CON			Male PB125			Female CON			Female PB125			Arbitrary units	Three Way ANOVA			
				Mean	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**					
			5mo	0.99999992	0.23115772	12	1.25295275	0.31956989	12	1.12621233	0.22878589	12	1.32893917	0.31560442	12		Age: 0.0250, Sex: 0.5069, Treatment: 0.0270, Age x Trt: <0.0001		Fig 7A	
			15mo	1.66123992	0.45560936	12	1.05320842	0.34607459	12	1.64818992	0.70433508	12	1.07813308	0.32795079	12					
	There is a significant interaction between Disease/Age and Treatment on HO-1 protein content in the gastrocnemius, but no main effect of any factor.	Homogenized gastrocnemius		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	Arbitrary units	Age: 0.1800, Sex: 0.9351, Treatment: 0.989, Age x Trt: <0.0001		Fig 7B	
			5mo	1	0.24490753	9	1.67908289	0.64069604	9	1.25892978	0.39100794	9	1.65851622	0.91520416	9					
			15mo	2.330179	1.10079732	9	1.13138967	0.69051276	9	2.15458922	1.28148039	9	1.00671233	0.60616972	9					
	There are no effects of			Male CON			Male PB125			Female CON			Female PB125				Three Way ANOVA			
				Mean	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**					

11. Are there Disease/Age-, Sex-, and Treatment related effects on carbonyl content in the soleus or gastrocnemius ?	Disease/Age, Treatment, or Sex, on carbonyl content in the soleus.There was a significant Sex x Age interaction	Homogenized soleus	**5mo**	1	0.25154744	6	1.092113	0.1105629	6	1.12699967	0.09041707	6	1.16333067	0.10674212	6	Arbitrary units	Age: 0.3032, Sex: 0.6274, Treatment: 0.9812, Age x Sex: 0.0069	Fig 7C
			15mo	1.20697333	0.08339618	6	1.211697	0.13721804	6	1.1378355	0.16632006	6	1.00069025	0.05576008	4			
	There are no effects of Disease/Age, Treatment, or Sex, on carbonyl content in the gastrocnemius.	Homogenized gastrocnemius		**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	Arbitrary units	Age: 0.0814, Sex: 0.9667, Treatment: 0.1179	Fig 7D
			5mo	1.2	0.32426597	5	1.74343667	1.28779756	6	1.16942083	0.42427076	6	2.93925883	1.81713991	6			
			15mo	2.81992767	1.08704093	6	2.4613865	0.94397067	6	1.9843642	0.86731134	5	2.07796683	0.99866852	6			
12. Does PB125 treatment slow the progression of disability in either male or female guinea pigs? (as defined by a cessation	PB125 does not slow the loss of mobility in male guinea pigs	Voluntary movement in an enclosed area.	Survival curve CON Males: 14 PB125 Males: 12						Chi square 0.05886			Median Survival Ratio (A/B) 1.176 95% CI 0.4127 – 3.354				Cessation of voluntary movement	Gehan-Breslow-Wilcoxon test CON vs PB125 P value 0.8083	Fig 7E
	PB125 does not slow the loss of mobility in female guinea pigs	Voluntary movement in an enclosed area.	Survival curve CON Females: 12 PB125 Females: 12						Chi square 0.05800			Median Survival Ratio (A/B) 0.5714 95% CI 0.2175 – 1.501				Cessation of voluntary movement	Gehan-Breslow-Wilcoxon test CON vs PB125 P value 0.4463	Fig 7F