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Autor*innen/Author(s): Misa Hirose, Paul Schilf, Yask Gupta, Marvin N. Wright, Marvin N. Wright, Olaf Jöhren, Anika E. Wagner, Christian Sina, Andreas Ziegler, Michael Ristow & Saleh M. Ibrahim

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Lifespan effects of mitochondrial mutations

ARISING FROM A. Latorre-Pellicer *et al. Nature* **535,** 561–565 (2016); doi:10.1038/nature18618

Somatic mitochondrial DNA (mtDNA) mutations accumulate within various tissues with age[1,2], however evidence directly showing the influence of mtDNA natural variations on ageing has been limited to date. Recently, Latorre-Pellicer *et al.* demonstrated that polymorphisms within mtDNA affect reactive oxygen species (ROS) levels, body mass, ageing score, tumour incidence and lifespan of conplastic mice[3]. Here we show that similarly generated conplastic strains, which carry a nuclear *Nnt* mutation, do not show any alterations in these parameters, demonstrating the relevance of specific mitonuclear interactions in determining mammalian healthspan through increased production of ROS.

Latorre-Pellicer *et al.*[3] compared a conplastic mouse strain that they developed from a C57BL/6JOlaHsd nuclear genome and NZB/OlaHsd mtDNA (BL/6^{NZB}) mice carrying various mutations in the mitochondrial genome with the original C57BL/6JOlaHsd carrying unaltered mtDNA (BL/6^{C57}). Despite the increased levels of ROS at a young age, BL/6^{NZB} mice showed a delayed ageing phenotype, including reduced tumour incidence culminating in an extended lifespan, which is consistent with previously published findings in invertebrates[4]. In this study[4], it was shown that metabolic induction of mitochondrial ROS formation promotes longevity in the nematode *Caenorhabditis elegans*, and that quenching this ROS signal by antioxidants abrogates the increase in lifespan.

C57BL/6J mice (Jackson Laboratories, JAX no. 000664) are known to harbour a mutation in a nuclear gene encoding the mitochondrially located nicotinamide nucleotide transhydrogenase (NNT) protein that renders the enzyme undetectable, resulting in reduced cytosolic antioxidant capacity and increased production of hydrogen peroxide[5] as well as impaired glucose tolerance[6], independent of any additional mitochondrial variation. We generated conplastic C57BL/6J-mt$^{NZB/BnlJ}$ (mtNZB/BlnJ)[7] and C57BL/6J (mtC57BL/6J) mice similar to the design used by Latorre-Pellicer *et al.*[3]. Notably, we did not observe an extension of the median or maximum lifespan of our conplastic mtNZB/BlnJ mice ($P=0.251$, log-rank test; $P=0.943$, Gehan test; Fig. 1a and Extended Data Table 1a–d) despite our large cohort size ($n=155$, mtC57BL/6J; $n=131$, mtNZB/BlnJ) resulting in high statistical power (>99% for the Gehan test, which places higher weight on early deaths; and as used by Latorre-Pellicer *et al.*[3]). Furthermore, we did not observe any differences in body mass, ageing score, tumour incidence (Fig. 1b–d) or spontaneous locomotor activity (Extended Data Fig. 1a, b). In addition, ROS levels, electron transport chain complex activity, and energy expenditure between our two strains showed no significant differences. An independent survival analysis with a log-rank test (which weights all subjects equally) also did not reveal any effect of the introduction of conplastic mtDNA on the lifespan of *Nnt*-deficient mice. Given that the published BL/6^{NZB} line and our mtNZB/BlnJ mice harbour essentially the same mtDNA mutations (Extended Data Table 2), the simplest interpretation for the different results obtained by Latorre-Pellicer *et al.*[3] and us is that the absence of NNT protein negates the effects of mitochondrial variation on healthspan. Together with the data from Latorre-Pellicer *et al.*[3], our findings indicate that mtDNA mutations that increase ROS levels on a functional NNT background are associated with an increased healthspan[3], whereas unaltered ROS levels prevent this effect on the progression of ageing (Fig. 1), both consistent with findings on mitohormesis[4]. As demonstrated in a variety of biological systems including humans[8], low-dose increases in mitochondrial ROS promote health and longevity, whereas higher doses cause

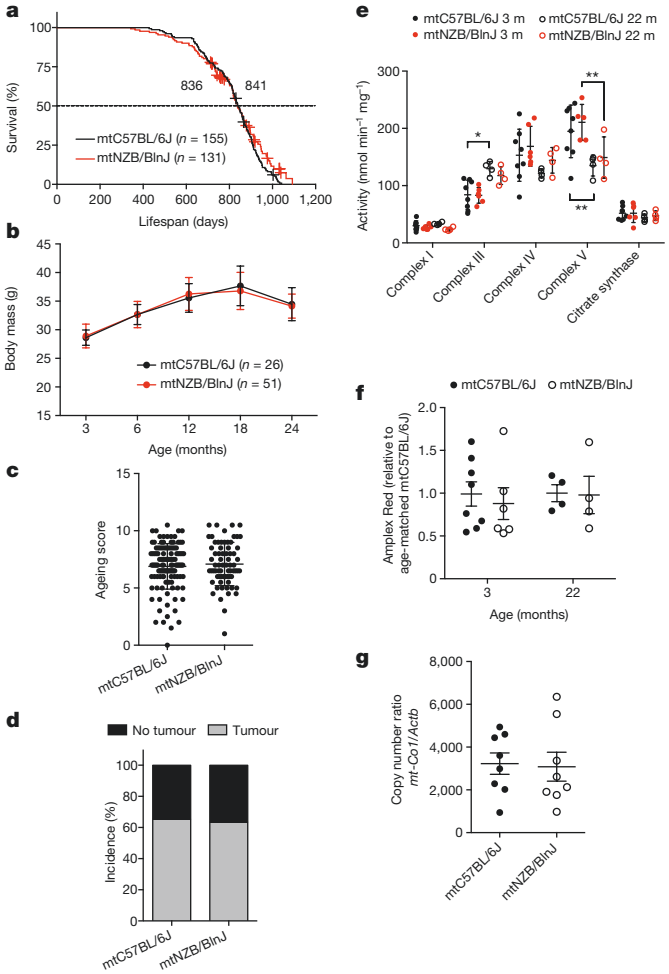

Figure 1 | Healthspan study in the conplastic strain C57BL/6J-mt$^{NZB/BlnJ}$ (mtNZB/BlnJ) and in C57BL/6J (mtC57BL/6J) control mice. **a**, Survival curves of mtC57BL/6J ($n=155$ (68 males and 87 females), median lifespan = 841 days) and mtNZB/BlnJ ($n=131$ (63 females and 68 males), median lifespan = 836 days) mice (log-rank test, $P=0.251$; Gehan test, $P=0.943$). Crosses indicate the censored animals (animals with censored survival time, that is, animals that were still alive at the time of analysis). **b**, Body mass (normal chow diet) of 26 mtC57BL/6J control mice and 51 mtNZB/BlnJ conplastic mice. **c**, Ageing score of mice at the moribund stage (mtC57BL/6J: 48 males and 70 females; mtNZB/BlnJ: 39 males and 31 females) (*t*-test, $P=0.4815$). **d**, Tumour incidence in moribund and spontaneously deceased mice (mtC57BL/6J: 49 males and 72 females; mtNZB/BlnJ: 39 males and 32 females) (Fisher's exact test, $P=0.8760$). **e**, Mitochondrial electron transport chain complex activities in isolated hepatic mitochondria ($n\geq5$ per genotype in 3-month-old mice (3 m); $n=4$ per genotype in 22-month-old mice (22 m)) (two-way ANOVA, $^{*}P=0.0197$ (mtC57BL/6J 3 m versus mtC57BL/6J 22 m), $^{**}P=0.0024$ (mtC57BL/6J 3 m versus mtC57BL/6J 22 m) and $^{**}P=0.0047$ (mtNZB/BlnJ 3 m versus mtNZB/BlnJ 22 m)). **f**, Mitochondrial ROS levels in the supernatant of isolated liver mitochondria normalized to age-matched mtC57BL/6J mice ($n=8$ mtC57BL/6J, 3 months; $n=6$ mtNZB/BlnJ, 3 months; $n=4$ per genotype at 22 months) (*t*-test, $P=0.6344$ at 3 months and $P=0.9330$ at 22 months). **g**, Ratios of mitochondrial DNA (*mt-Co1*) to nuclear DNA (*Actb*) copy numbers in genomic DNA of hepatocytes ($n=8$ per genotype at 3 months) (*t*-test, $P=0.8641$).

the opposite effect by causing cellular and systemic damage, reflecting a nonlinear, that is, hormetic, response to a mitochondrial stressor, namely ROS[9,10].

The median and maximum lifespans in Latorre-Pellicer *et al.*[3] are reduced compared with those in our study (published median lifespan in BL/6^{C57}, 741 days; our median in mtC57BL/6J, 841 days). The reported lifespan of Jackson Laboratory C57BL/6J mice without *Nnt* is longer than the C57BL/6JNNia mice with the *Nnt* gene. However, other differences that are known to affect lifespan, such as environmental influences (including housing conditions, diet, handling and microbiota), cannot be excluded at present.

In summary, we consider the use of conplastic animals an important approach for the investigation of the putative effect of mtDNA on mammalian physiology. Nevertheless, and besides the potential impact of environmental conditions, we assume that the pronounced differences outlined here can be largely attributed to the *Nnt* mutation in the nuclear genome of the Jackson C57BL/6J sub-strain used here. Accordingly, further experimental studies involving the same and additional strains should be performed to study the impact of mtDNA variations and mitochondrial ROS signalling to increase our knowledge of the related pathways responsible for the control of mammalian healthspan.

Misa Hirose[1], Paul Schilf[1], Yask Gupta[1], Marvin N. Wright[2], Olaf Jöhren[3,4], Anika E. Wagner[5], Christian Sina[5], Andreas Ziegler[2,6,7], Michael Ristow[8] & Saleh M. Ibrahim[1]

[1]Lübeck Institute of Experimental Dermatology, University of Lübeck, Germany.
email: saleh.ibrahim@uksh.de
[2]Institute of Medical Biometry and Statistics, University of Lübeck, University Medical Center Schleswig-Holstein, Campus Lübeck, Germany.
[3]Institute of Experimental and Clinical Pharmacology and Toxicology, University of Lübeck, Lübeck, Germany.
[4]Center of Brain, Behavior and Metabolism (CBBM), University of Lübeck, Lübeck, Germany.
[5]Institute of Nutritional Medicine, University of Lübeck, Lübeck, Germany.
[6]ZKS Lübeck, University of Lübeck, Lübeck, Germany.
[7]School of Mathematics, Statistics and Computer Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa.
[8]Energy Metabolism Laboratory, Swiss Federal Institute of Technology (ETH) Zurich, Schwerzenbach, Switzerland.

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Author Contributions M.H. and S.M.I. designed the study. M.H., P.S. and C.S. performed the experiments and analysed the data. M.W. and A.Z. conducted the statistical analysis of the survival data. Y.G. analysed the sequencing data. O.J. performed the indirect calorimetric cage experiment and analysed the data. M.H., A.E.W., M.R. and S.M.I. wrote the manuscript with contributions from all other authors. S.M.I. directed the study.

Competing Financial Interests Declared none.

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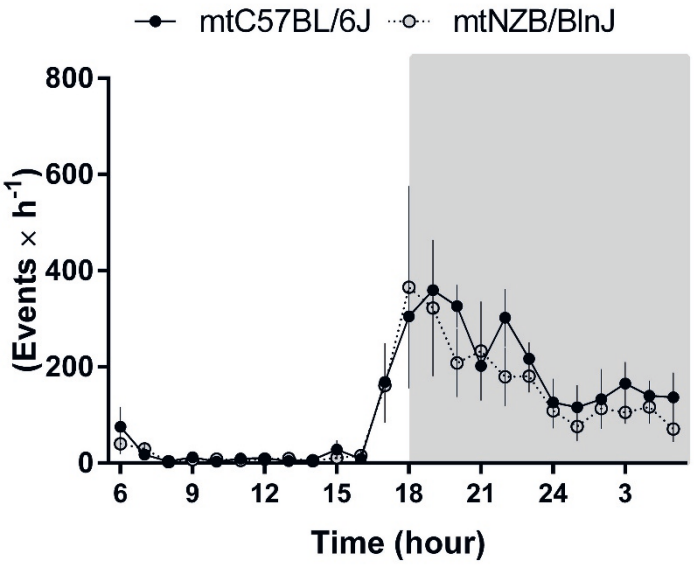
a.



b.

Area under activity curves

Gender	Strain	Period			
		Day		Night	
		Value	*p*	Value	*p*
Female	mtC57BL/6J	223.6	0.7746	2311	0.4779
	mtNZB/BlnJ	202.2		1864	

c.

Respiratory parameter

Gender	Strain	RER	EE
Female	mtC57BL/6J	20.26±0.22	278.1±11.40
	mtNZB/BlnJ	20.20±0.07	288.3±7.98

Extended Data Figure 1 | Indirect calorimetric cage analysis of mtC57BL/6J and mtNZB/BlnJ mice. a, Spontaneous locomotor activities. **b**, Area under the curve analysis of the activities shown in **a**. **c**, Area under the curve of respiratory exchange ratio (RER) and energy expenditure (EE). Data were obtained from 5 female mtC57BL/6J mice and 6 female mtNZB/BlnJ mice.

Extended Data Table 1 | Statistical analysis for the lifespan of mtC57BL/6J and mtNZB/BlnJ mice

a. Statistical analysis for survival curves; mtC57BL/6J versus mtNZB/BlnJ.

	Log-rank	Gehan
Total	0.251	0.946
Female	0.188	0.819
Male	1.000	0.304

b. Median survival times by sex.

	N	Events	Median	95% confidence interval
Female	150	125	822	789 - 844
Male	136	121	864	838 - 887

c. Median survival times by strain.

	N	Events	Median	95% confidence interval
mtC57BL/6J	155	151	841	823 - 864
mtNZB/BlnJ	131	95	836	818 - 875

d. Median survival times by strain and sex.

		N	Events	Median	95% confidence interval
mtC57BL/6J	Female	87	84	817	781 - 848
	Male	68	67	871	842 - 904
mtNZB/BlnJ	Female	63	41	836	775 - 932
	Male	68	54	851	812 - 888

Extended Data Table 2 | Sequence difference between mtDNA of mtNZB/BlnJ and mtC57BL/6J mice

Position	C57BL/6J	NZB/BlnJ	Gene	Type of mutation	Position	C57BL/6J	NZB/BlnJ	Gene	Type of mutation
55	G	A	*mt-Tf*	non-coding	8858	T	C	*mt-Co3*	synonymous
1353	A	G	*mt-Rnr2*	non-coding	8864	C	T	*mt-Co3*	synonymous
1519	G	A	*mt-Rnr2*	non-coding	9137	A	G	*mt-Co3*	synonymous
1590	G	A	*mt-Rnr2*	non-coding	9152	T	C	*mt-Co3*	synonymous
1822	T	C	*mt-Rnr2*	non-coding	9391	A	G	*mt-Tg*	non-coding
2201	T	C	*mt-Rnr2*	non-coding	9461	T	C	*mt-Nd3*	synonymous
2340	G	A	*mt-Rnr2*	non-coding	9530	C	T	*mt-Nd3*	synonymous
2525	C	T	*mt-Rnr2*	non-coding	9581	C	T	*mt-Nd3*	synonymous
2766	A	G	*mt-Nd1*	non-synonymous	9599	A	G	*mt-Nd3*	synonymous
2767	T	C	*mt-Nd1*	non-synonymous	9820	8A	10A	*mt-Tr*	non-coding
2798	C	T	*mt-Nd1*	synonymous	9985	G	A	*mt-Nd4l*	non-synonymous
2814	T	C	*mt-Nd1*	synonymous	10547	C	T	*mt-Nd4*	synonymous
2840	C	T	*mt-Nd1*	synonymous	10583	A	G	*mt-Nd4*	synonymous
2934	C	T	*mt-Nd1*	non-synonymous	10952	C	A	*mt-Nd4*	synonymous
3194	T	C	*mt-Nd1*	synonymous	11843	G	A	*mt-Nd5*	synonymous
3260	A	G	*mt-Nd1*	synonymous	11846	C	T	*mt-Nd5*	synonymous
3422	T	C	*mt-Nd1*	synonymous	11933	A	C	*mt-Nd5*	synonymous
3467	T	C	*mt-Nd1*	synonymous	12353	C	T	*mt-Nd5*	synonymous
3599	T	C	*mt-Nd1*	synonymous	12575	T	A	*mt-Nd5*	synonymous
3692	A	G	*mt-Nd1*	synonymous	12695	A	G	*mt-Nd5*	synonymous
3932	G	A	*mt-Nd2*	non-synonymous	12835	T	C	*mt-Nd5*	non-synonymous
4123	C	T	*mt-Nd2*	synonymous	12890	A	G	*mt-Nd5*	synonymous
4276	G	A	*mt-Nd2*	synonymous	13004	G	A	*mt-Nd5*	synonymous
4324	T	C	*mt-Nd2*	synonymous	13444	C	T	*mt-Nd5*	non-synonymous
4408	G	A	*mt-Nd2*	synonymous	13612	T	C	*mt-Nd6*	synonymous
4706	A	G	*mt-Nd2*	non-synonymous	13689	C	T	*mt-Nd6*	non-synonymous
4732	C	T	*mt-Nd2*	synonymous	13781	A	G	*mt-Nd6*	non-synonymous
4771	T	C	*mt-Nd2*	synonymous	13782	T	C	*mt-Nd6*	non-synonymous
4885	A	C	*mt-Nd2*	synonymous	13837	A	G	*mt-Nd6*	synonymous
4903	T	G	*mt-Nd2*	synonymous	13983	A	G	*mt-Nd6*	synonymous
5204	A	AG	*mt-Tc*	non-coding	14186	T	C	*mt-Cytb*	synonymous
5463	G	A	*mt-Co1*	non-synonymous	14211	G	A	*mt-Cytb*	non-synonymous
5552	T	C	*mt-Co1*	synonymous	14363	A	G	*mt-Cytb*	synonymous
5930	G	A	*mt-Co1*	synonymous	14642	G	A	*mt-Cytb*	synonymous
6041	T	C	*mt-Co1*	synonymous	14738	C	T	*mt-Cytb*	synonymous
6407	C	T	*mt-Co1*	synonymous	15499	T	A	D-loop	intergenic
6470	A	G	*mt-Co1*	synonymous	15549	C	T	D-loop	intergenic
6575	C	T	*mt-Co1*	synonymous	15578	A	T	D-loop	intergenic
6620	G	A	*mt-Co1*	synonymous	15588	C	T	D-loop	intergenic
6785	G	A	*mt-Co1*	synonymous	15603	C	T	D-loop	intergenic
7411	A	G	*mt-Co2*	synonymous	15657	T	C	D-loop	intergenic
7546	A	G	*mt-Co2*	synonymous	15917	C	T	D-loop	intergenic
7870	G	A	*mt-Atp8*	synonymous	16017	A	C	D-loop	intergenic
8439	A	G	*mt-Atp6*	synonymous	16268	A	G	D-loop	intergenic
8467	T	C	*mt-Atp6*	synonymous	16272	T	C	D-loop	intergenic
8568	C	T	*mt-Atp6*	synonymous					

Heteroplasmy at nt7546 (50–80%, synonymous, *mt-Co2*) and non-synonymous mutation at nt9985 (*mt-Nd4l*) were not observed in the study by Latorre-Pellicer *et al.*[3] In addition, the A > G mutation at nt716 (*mt-Rnr2*) was not found in our study.