

1 **Reduced insulin and IGF-1 signalling synergistically extend healthspan in male**  
2 **mice**

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1 **Abstract**

2 Reduced IGF-1 signalling is an evolutionarily conserved mediator of longevity, yet the  
3 magnitude of this effect is substantially larger in organisms retaining a common insulin  
4 and IGF-1 receptor. Whether this discrepancy reflects the failure to simultaneously  
5 reduce IGF-1 *and* insulin signalling in mammalian model systems remains unexplored.  
6 Moreover, studies of invertebrates cannot ascertain whether substantial effects upon  
7 lifespan are associated with preserved cognitive performance, a crucial component of  
8 healthspan. We compared the healthspan of male mice with haploinsufficiency of the  
9 insulin receptor (IRKO), IGF-1 receptor (IGF-1RKO), or both (DKO), with wildtype  
10 (WT) littermates. DKO mice survived longer than WT, with IRKO and IGF-1RKO being  
11 intermediate. At 2 years of age, DKO also exhibited preserved nesting behaviour in  
12 contrast with all other genotypes. Differential insulin sensitivity or weight gain during  
13 ageing did not explain the preserved healthspan of DKO, since these were comparable  
14 to IRKO littermates. These data provide the first demonstration that reduced insulin  
15 and IGF-1 signalling have synergistic effects upon healthspan in a mammalian model  
16 system, suggesting future mechanistic and translational studies should target insulin  
17 and IGF-1 signalling.

## 1 **Introduction**

2 The association between reduced insulin/IGF-1 signalling and longevity has been  
3 established in diverse model organisms using genetic, pharmacological and dietary  
4 interventions (1), prompting interest in this as a paradigm to extend human lifespan.  
5 However, the striking observations made in genetically modified invertebrates, which  
6 share a common insulin and IGF-1 receptor, have been subtler in mammalian model  
7 systems with isolated targeting of insulin or IGF-1 receptors (2). Whether these  
8 discrepancies reflect a failure to simultaneously target the functionally overlapping  
9 insulin and IGF-1 signalling apparatus remains unknown, and is an important barrier  
10 to developing effective strategies to promote healthy ageing. Moreover, it is  
11 increasingly appreciated that extension of lifespan may come at the expense of  
12 extending time with poor health, resulting in a focus on interventions that prolong  
13 healthy life, or healthspan (3). The literature describing whether reduced insulin and  
14 IGF-1 signalling protects against ageing-associated functional decline is sparse,  
15 particularly when applied to genetic interventions in mammalian models. Hence, we  
16 set out to study whether reduced insulin and/or IGF-1 receptor expression extend  
17 healthy life in mice.

18

## 19 **Materials and methods**

20 *Acquisition, breeding and husbandry of mice:* Mice were bred onto a C57BL/6J  
21 background for >10 generations in a conventional animal facility with 12-hour light/dark  
22 cycle. A standard chow diet (Beekay BK001E, B&K Universal Limited) was provided,  
23 which contained 4.7% fat, 18.7% protein and 59.7% nitrogen free extract (16.3KJ/g).  
24 As previously described (4), male insulin receptor halpoinufficient mice (IRKO) were  
25 crossed with female IGF-1 receptor halpoinufficient mice (IGF-1RKO), resulting in

1 progeny with the following genotypes: 1) Wild-type (WT); 2) insulin receptor  
2 halpoinsufficient (IRKO); 3) IGF-1 receptor halpoinsufficient; and 4) insulin and IGF-1  
3 receptor halpoinsufficient (DKO). 15 male mice per genotype were observed during  
4 assessment of healthspan. All procedures were performed according to accepted  
5 standards of humane animal care, approved by the ethical review committee of the  
6 University of Leeds, and conducted under license from the United Kingdom Home  
7 Office.

8  
9 *Metabolic assessment:* Whole capillary blood was sampled from tail vein, with glucose  
10 concentrations determined in whole blood by a portable meter (Roche Diagnostics,  
11 UK). Glucose and insulin tolerance tests were performed by blood sampling after an  
12 intraperitoneal injection of glucose (1 mg/g; Sigma-Aldrich, UK) or human recombinant  
13 insulin (0.75 units/kg, Actrapid; Novo Nordisk, Denmark), respectively (4).

14  
15 *Healthspan endpoints:* Assessment of healthspan was made according to criteria  
16 provided by a Home Office approved Veterinary Surgeon, based upon published  
17 literature (5), to ensure animal welfare throughout the study. Animals were considered  
18 to have reached their healthspan endpoint if one or more of the following conditions  
19 was met: 1) Spontaneous death before one of the following endpoints; 2) Body  
20 condition score  $\leq 2$  out of 5; 3) Body weight loss of  $\geq 15\%$  of the average highest body  
21 weight, sustained for at least two consecutive weeks; 4) Hunched posture/starry  
22 coat/abnormal gait of more than 48 hours duration; 5) Any progressively enlarging  
23 subcutaneous lump/swelling; 6) Excessive hair loss, monitored over at least one week.  
24 Assessment to confirm whether an animal had met a healthspan endpoint was made  
25 by two independent observers except in the case of spontaneous death or body weight

1 loss of  $\geq 15\%$  of the average highest body weight, which were considered independent  
2 of inter-observer variability. Animals were culled in accordance with Schedule 1 of The  
3 Animals (Scientific Procedures) Act 1986 (Amended 2012) once a healthspan  
4 endpoint was reached. In keeping with our United Kingdom Home Office Project  
5 License (P144DD0D6) stipulations, any animals considered to be experiencing  
6 excessive pain or distress (outside of the criteria mentioned above) were culled after  
7 assessment by two independent observers blinded to genotype.

8

9 *Nesting studies:* Mice were caged individually and left overnight with a nestlet. The  
10 next morning the cage was examined for the presence of a nest and images taken to  
11 quantify nest building, according to an established validated protocol (6). Nest  
12 photographs were taken by a blinded researcher, and subsequently scored by 4  
13 genotype-blinded researchers per mouse, to derive a mean nesting score for each  
14 mouse. Scoring criteria were as follows: 1) Nestlet not noticeably touched (more than  
15 90% intact); 2) Nestlet partially torn (50–90% remaining intact); 3) Nestlet mostly  
16 shredded but often no identifiable nest site: less than 50% of the Nestlet remains  
17 intact, but less than 90% is within a quarter of the cage floor area; i.e., the cotton is  
18 not gathered into a nest but is spread around the cage. The material may sometimes  
19 be in a broadly defined nest area, but the critical definition here is that 50–90% has  
20 been shredded; 4) An identifiable but flat nest: more than 90% of the Nestlet is torn  
21 and the material is gathered into a nest within a quarter of the cage floor area, but the  
22 nest is flat, with walls higher than mouse body height (of a mouse curled up on its side)  
23 for less than 50% of its circumference; 5) A (near) perfect nest: more than 90% of the  
24 Nestlet is torn and the nest is a crater, with walls higher than mouse body height for  
25 more than 50% of its circumference.

1

2 *Statistics:* Data are presented as mean  $\pm$  SEM. All genotypes were compared with  
3 ANOVA or Kruskal-Wallis tests, as appropriate, with *post hoc* comparisons made  
4 using t-tests or Mann-Whitney U tests. Statistical significance was defined as  $p < 0.05$ .

5

## 6 **Results**

7 As previously described (4), we bred insulin receptor halpainsufficient mice with IGF-  
8 1 receptor halpainsufficient mice, producing progeny with the following genotypes:  
9 wild-type (WT); insulin receptor halpainsufficient (IRKO); IGF-1 receptor  
10 halpainsufficient (IGF-1RKO); insulin and IGF-1 receptor halpainsufficient (DKO).  
11 Male littermates ( $n=15$ /genotype) were then fed a standard chow diet and observed  
12 by researchers blinded to genotype until spontaneous death or an a priori defined  
13 humane endpoint described earlier. All genotypes gained weight during adulthood  
14 (Figure 1a), with mean weight at 18 months of age being significantly less in IRKO and  
15 DKO than WT and IGF-1RKO littermates (Figure 1b). At 20 months of age, this was  
16 associated increased glucose tolerance (Figure 1c), and increased insulin sensitivity  
17 (Figure 1d) in all surviving IRKO and DKO versus WT and IGF-1RKO littermates.  
18 Notably, body mass across genotypes correlated with glucose tolerance ( $R^2 = 0.48$ ;  
19 Figure 1e) and insulin sensitivity ( $R^2 = 0.31$ ).

20

21 Nesting studies were then performed in all mice surviving to 24 months of age, as a  
22 marker of behaviour and global cognitive performance. The mean nesting quality  
23 score allocated by 4 blinded assessors using a validated methodology (6) was  
24 significantly different between genotypes, with DKO exhibiting clearly superior  
25 performance against other groups (Figure 2a). Notably, nesting performance did not

1 correlate with body mass, and nesting scores in a subgroup of 3-month old mice from  
2 this colony demonstrated that all genotypes produced high quality nests (Figure 2b).  
3 Importantly, the superior nesting scores of DKO were also associated with extended  
4 survival free from markers of ill health that mandated euthanasia according to our  
5 humane endpoint protocol (Log rank  $p=0.04$  across all genotypes; Figure 2c). When  
6 comparing individual genotypes, only DKO survived significantly longer than WT (Log  
7 rank  $p=0.004$ ; median survival 868 versus 712 days), with survival of IRKO and IGF-  
8 1RKO groups being intermediate (median survival of 783 and 760 days, respectively).  
9

## 10 **Discussion**

11 Our study shows for the first time that genetically reduced insulin and IGF-1 signalling  
12 extends healthspan and retards cognitive decline in male mice, suggesting that  
13 observations made in invertebrates may be relevant to mammalian ageing. Notably,  
14 studies linking reduced insulin or IGF-1 signalling to murine longevity and stress  
15 resistance have found sexual dimorphism (7–12); hence it will be very important for  
16 future studies to examine female DKO mice, rather than generalising the differences  
17 we have observed in male mice. A striking observation from our data is that isolated  
18 reduction in insulin or IGF-1 signalling is insufficient to significantly extend healthspan  
19 parameters in male mice; this suggests a synergistic effect, possibly reflecting  
20 functional compensation between these evolutionarily related receptors (13).  
21 Moreover, our metabolic characterisation suggests that reduced body mass and  
22 increased insulin sensitivity, two parameters often associated with longevity (14), are  
23 not sufficient to denote healthy ageing, since the similar metabolic phenotype of IRKO  
24 and DKO was not mirrored in their healthspan. In summary, our data may reconcile  
25 conflicting observations from evolutionarily distant models of ageing, by emphasising

1 the enduring synergism between insulin and IGF-1 signalling. Future studies should  
2 address the molecular basis of this synergism, which may inform the development of  
3 more effective therapeutic approaches to extend healthy life.

4

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6 clinical research training fellowships. MTK is a BHF professor and RMC is a BHF  
7 intermediate clinical fellow.

8

## 9 **References**

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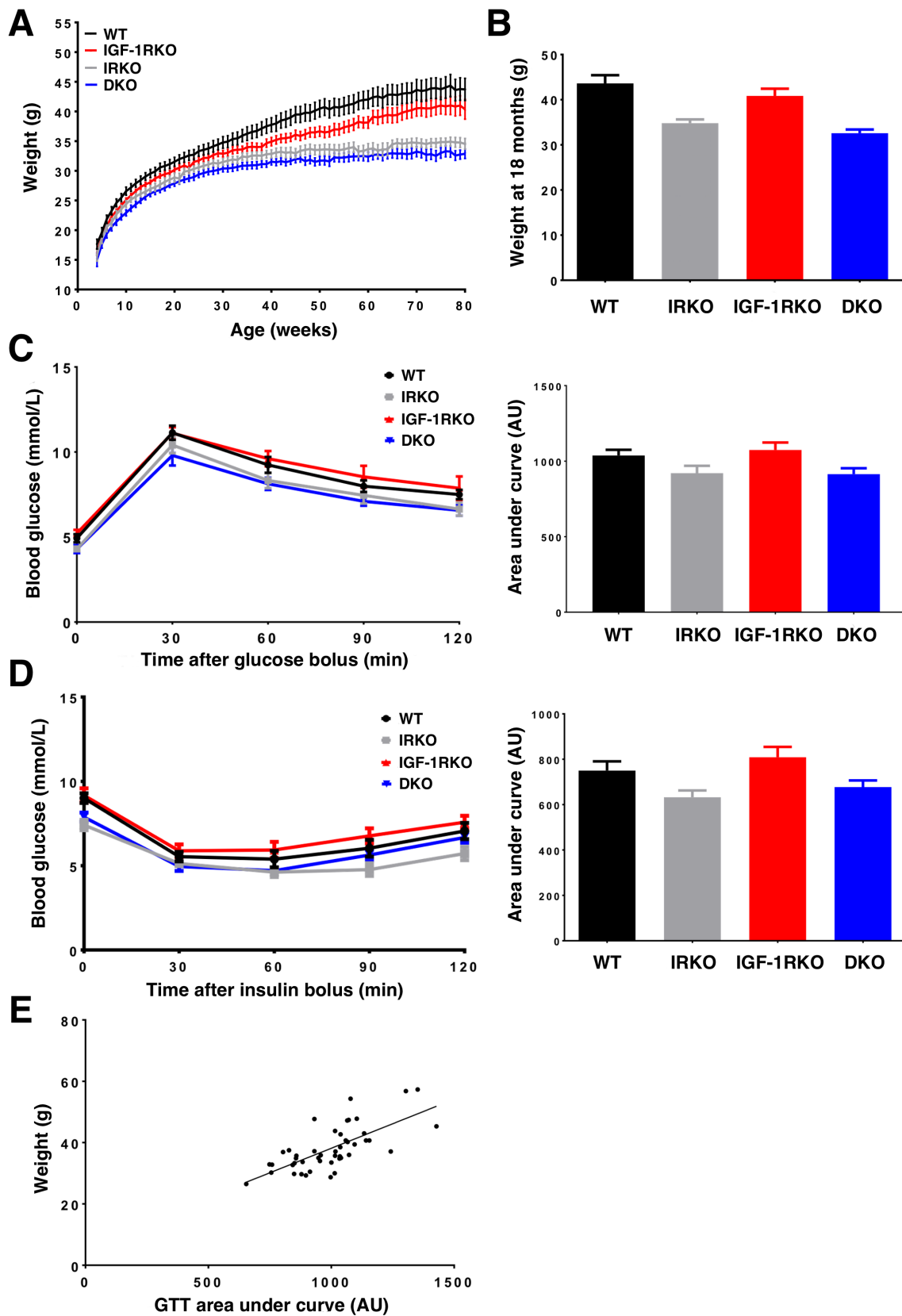
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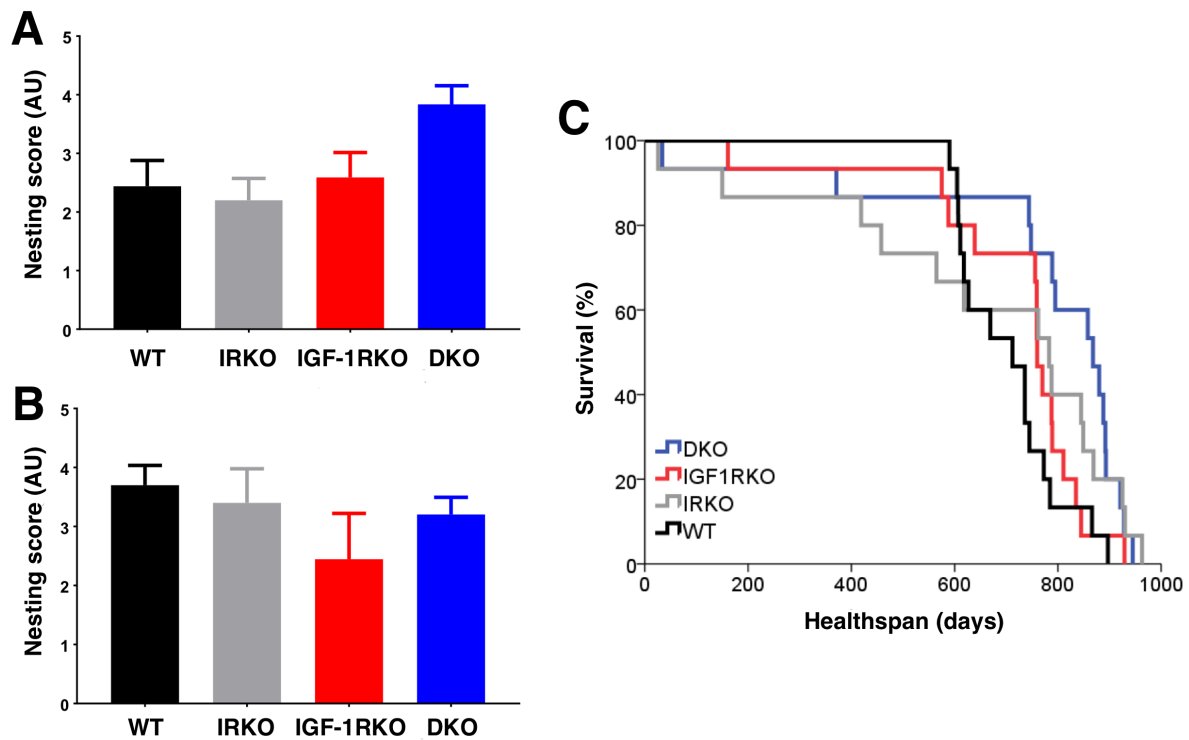
# 1 Figure 1: Metabolic characterisation during aging



2

1 A) Body mass during ageing (n=15/genotype); B) Body mass at 18 months (ANOVA  
2  $p < 0.001$ ; n=15,11,14,13); C) Glucose tolerance testing at 20 months, quantified by  
3 area under curve (ANOVA  $p = 0.03$ ; n=10,10,13,13); D) Insulin tolerance testing at 20  
4 months, quantified by area under curve (ANOVA  $p = 0.01$ ; n=11,10,13,13); E)  
5 Correlation between area under glucose tolerance test curve and body mass  
6 ( $p < 0.001$ ; n=46). AU – arbitrary units.

1 **Figure 2: Healthspan is extended in DKO mice**



2

3 A) Mean nesting score at 24 months (Kruskal-Wallis  $p=0.01$ ;  $n=4,5,7,11$ ); B) Mean  
4 nesting score at 3 months (Kruskal-Wallis  $p=0.42$ ;  $n=5,3,3,9$ ); C) Kaplan-Meier curve  
5 illustrating healthspan (Log rank  $p=0.04$ ;  $n=15$ /genotype). AU – arbitrary units.