Effects of pre and post natal diet on survival of SMA mouse model SMNΔ7

Rachel M. Sinnott, John T. McCrone, Frankie Rose, Christian L. Lorson

ABSTRACT & INTRODUCTION

Spinal muscular atrophy (SMA) is the leading genetic cause of infantile death. SMA is an autosomal recessive disease caused by the loss of or mutation in survival motor neuron (SMN) 1 gene. This results in a significant reduction in SMN protein levels and death of α-motor neurons. Humans have SMN2, an almost identical gene. The only significant difference between SMN1 and 2 is a C to T switch in exon 7 which causes alternative mRNA splicing. Only 10 – 20% of SMN2 transcripts result in full length SMN proteins.

SMN Δ7 SMA mouse model is a mSmn knockout with a transgenically inserted SMN2 with a mutation in exon 7, mimicking the human SMN2. The transgenic SMN Δ7 SMA mouse model lives ~ 13 to 18 days on average. Previous studies indicate one explanation for the variation in lifespan is diet. Diet has also been shown to affect the SMA phenotype. Pico diet extends the lifespan by ~25% compared to the Harlan diet. One major difference between the two diets is fat content- Pico having 9% and Harlan having 5%.

In this study, we're looking at pre and post-natal dietary affects on lifespan and weight of affected SMN Δ7 pups. Litters were divided into 4 groups- 2 maintained the same diet through the study (either high or low fat) and 2 had their diets switched (high to low fat and vice versa). Pups were genotyped; only homozygous unaffected and affected were considered. The purpose is to figure out when in development nutrition, ie. fat content, becomes a factor in the SMA phenotype. Our results indicate that a post natal switch from low to high fat diet results in ~5% increase in lifespan. This information may impact therapeutic testing in mice models as well as dietary modifiers of the SMA phenotype.

BACKGROUND

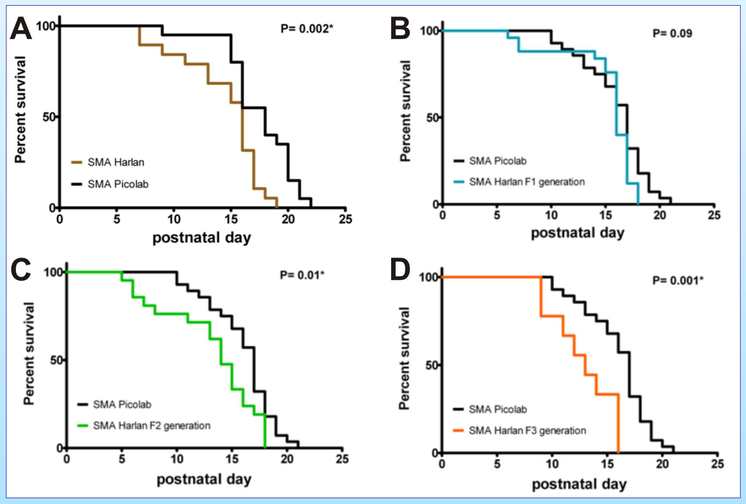
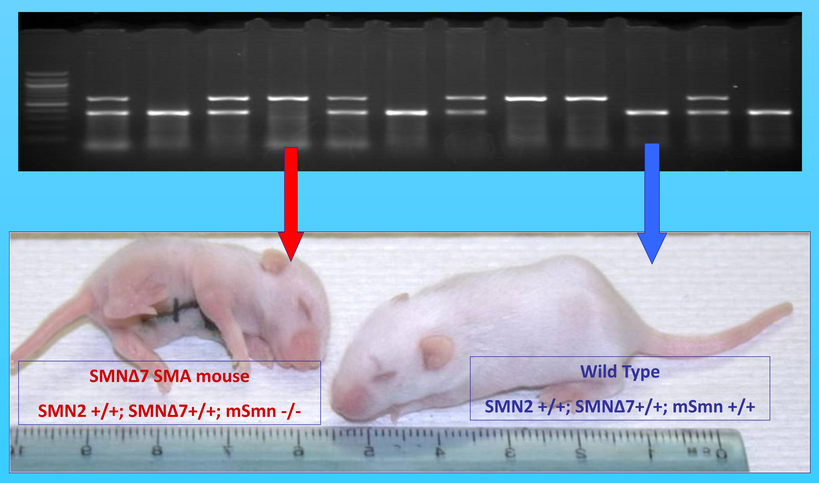


Fig 1: effects of diet on SMN Δ7 SMA mouse model.
-A: SMA pups from a dam transitioned from the Harlan diet → PicoLab diet
-B → D: SMA pups transitioned from Pico → Harlan for 1 (B), 2 (C) & 3 (D) generations
- SMN Δ7 SMA mice on the PicoLab diet had a significantly longer average lifespan than those on the Harlan diet

METHODS

- Male and female genotype **SMN2 +/+; SMNΔ7+/+; mSmn +/-** were crossed to generate SMNΔ7 SMA mice **SMN2 +/+; SMNΔ7+/+; mSmn -/-**
- Mice were divided into 4 groups based on diet:
  - -1: biological mother fed high fat diet (Pico) → post natal mother fed low fat diet (Harlan)
  - -2: no switch – low fat
  - -3: biological mother fed low fat diet → post natal mother fed high fat diet
  - -4: no switch – high fat
- Neonates were genotyped via PCR assays on genomic DNA from tail biopsies
- Only homozygous wild type and affected were followed through the study (fig)
- Mice were weighed daily and their lifespans recorded



RESULTS

Survival Curves Comparing SMA Pups From All 4 Groups

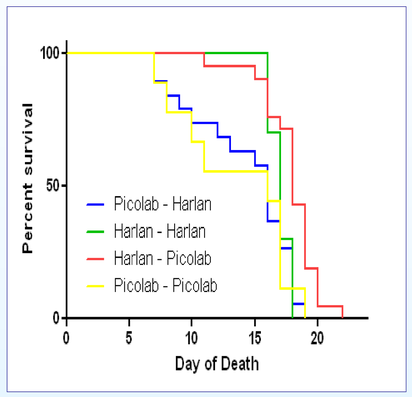


Fig 2: Contrary to our expectations, these graphs imply that those mice starting (and continuing) on the Harlan diet tend to live longer than those on the PicoLab diet. This is most likely due to the different genetic backgrounds of the mice. The SMA mouse model is not congenic and therefore is a mixture of two different inbred genetic backgrounds (~90% FVB/NJ and ~ 10% C57BL/6J).

Mice starting out on the Harlan diet were fed the Harlan diet for 7 generations. Mice starting on the Pico lab diet were taken from populations that had been fed the Pico diet for several generations. We can only compare mice from the same genetic background for significant data (fig 3).

Survival curve comparison of Harlan background mice maintained on the low-fat diet versus those switched postnatally to a high- fat diet

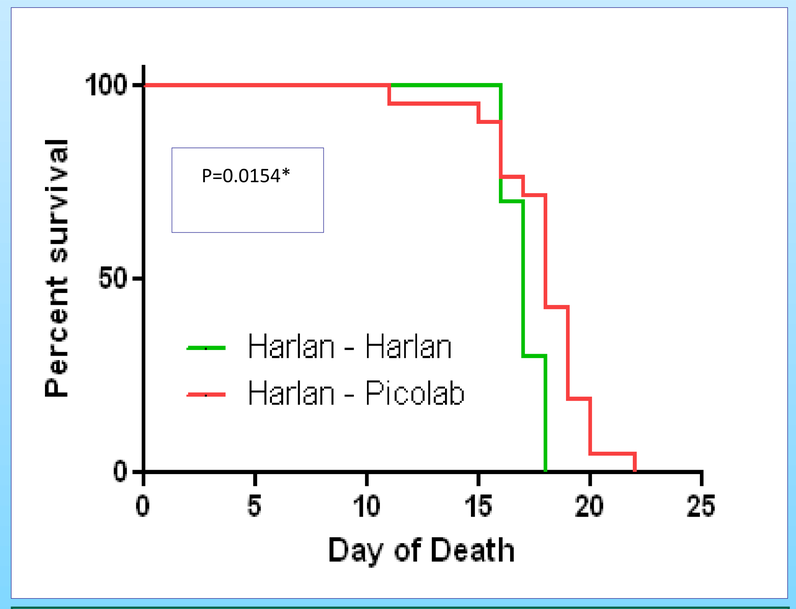


Fig 3: Mice switched postnatal to a high fat diet tend to live longer (P value of 0.0154).

Weights of affected pups

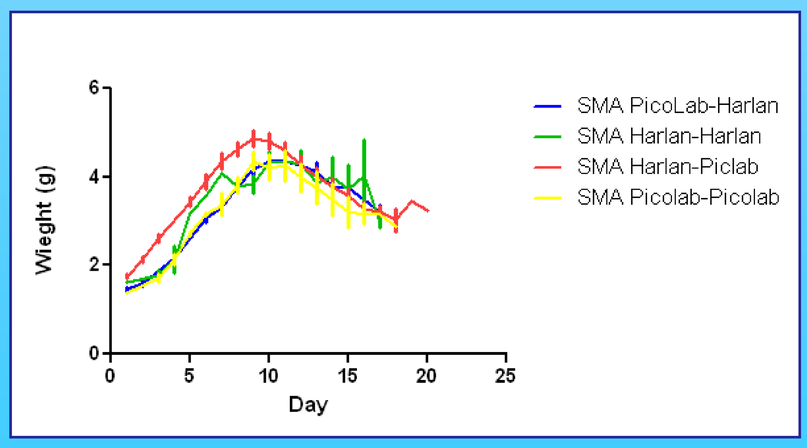


Fig 4: Affected pup weight tends to peak and then drop off before death. It is not known how much of this weight loss is due to loss of muscle mass due to alpha motor neuron loss and muscle atrophy versus how much is due to starvation.

CONCLUSION

Results (fig 3) show there is a significant difference in the survival curves between affected pups maintained on the Harlan diet versus those switched to the PicoLab diet postnatal. There appears to be a quicker drop-off in surviving pups maintained on the Harlan diet.

The average lifespan of groups 2 and 3 were 17 and 18.25 respectively. Group 3 contained one outlier value which was disregarded in the t test comparing the average lifespans of the two groups. Average lifespans were found to be statistically different at the 95% confidence level. Overall these results suggest that a postnatal switch in diet alone can significantly increase the lifespan of these SMA delta 7 knockout pups. Because fat content is the only significant difference between the two diets, results suggest that increasing fat is the main factor for the difference in survival curves and lifespan. However, more research is needed to say conclusively that differences in fat is the only factor that could cause this shift in lifespan (in this study, we used two commonly used lab rodent diets).

This study reemphasizes the importance of standardizing model conditions for accurate and comparable results, especially between collaborating lab groups. More research is required to determine if there is any therapeutic benefit to a high fat diet in SMA cases.

FUTURE RESEARCH

- Does increasing dietary fat alone lengthen the lifespan of Δ7 SMA model affected pups?
  - Could an increase in calories alone is able to sustain them longer?
- Is myelination impaired? If so, increasing fatty acids in the diet may provide essential building blocks for myelination.
- It's still unknown what specifically causes death in the SMA model mice. Affected people die from respiratory complications due to loss of function in their intercostal muscles, for example. However, starvation may also be a factor in mice. Affected pups may not be able to compete with littermates for food. If that were the case, a higher caloric diet would extend their lifespan.

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- 2nd SMA mouse photo from http://www.NIH.gov