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Short report

Increased lifespan in hyposulfatemic NaS1 null mice

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ABSTRACT

Sulfate (SO_4^{2-}) plays an important role in mammalian growth and development. In this study, hyposulfatemic NaS1 null (*Nas1*−/−) mice were used to investigate the consequences of perturbed SO_4^{2-} homeostasis on longevity. Median life spans were increased (by ≈25%) in male and female *Nas1*−/− mice when compared with *Nas1*+/+ mice. At 1 yr of age, serum SO_4^{2-} levels remained low in *Nas1*−/− mice (≈0.16 mM) when compared to *Nas1*+/+ mice (≈0.96 mM). RT-PCR revealed increased hepatic mRNA levels of *Sirt1* (by ≈60%), *Cat* (by ≈48%), *Hdac3* (by ≈22%), *Trp53* and *Cd55* (by ≈36%) in *Nas1*−/− mice, genes linked to ageing. Histological analyses of livers from 2 yr old mice revealed neoplasms in >50% of *Nas1*+/+ mice but not in *Nas1*−/− mice. This is the first study to report increased lifespan, decreased hepatic tumours and increased hepatic expression of genes linked to ageing in hyposulfatemic *Nas1*−/− mice, implicating a potential role of SO_4^{2-} in mammalian longevity and cancer.

1. Introduction

SO_4^{2-} is essential for numerous metabolic and cellular processes (Markovich, 2001). SO_4^{2-} conjugation (sulfonation) activates dietary compounds such as heterocyclic amines to toxic intermediates which are implicated in carcinogenesis (Glatt et al., 2000). Circulating SO_4^{2-} levels are maintained by the NaS1 sulfate transporter, which is expressed in the kidney where it mediates SO_4^{2-} reabsorption (Beck and Markovich, 2000; Lee et al., 2000; Markovich et al., 1993). NaS1 null (*Nas1*−/−) mice generated in our lab exhibit hyposulfatemia, growth retardation and reduced circulating IGF-1 levels (Dawson et al., 2003), which has been implicated in longevity (Longo and Finch, 2003). The NaS1 gene (*Slc13a1*) belongs to the same Slc13 gene family (Markovich and Aronson, 2007; Markovich and Murer, 2004) as *Indy* which has been linked to increased lifespan in *D. melanogaster* (Rogina et al., 2000). These findings led us to investigate the lifespan of *Nas1*−/− and *Nas1*+/+ mice to determine if hyposulfatemia has an effect on longevity.

2. Materials and methods

2.1. Mice

Nas1−/− mice used in this study were previously generated by targeted disruption of the mouse Nas1 gene (Dawson et al., 2003). The breeding strategy used was hemizygous Nas1 ±/− crosses. Survival of mice was monitored on a daily basis and grouped into monthly age intervals (Supplemental Table 1). Mice were housed 2 to 5 per cage in conventional conditions, fed a standard rodent chow (no. AIN93G: Glen Forrest Stockfeeders, Glen Forrest, Western Australia) and water *ad libitum*, and were used for approved experiments in accordance with the guidelines of the University of Queensland Animal Ethics Committee.

2.2. Sulfate assays

A modified turbidometric assay was used to measure free sulfate levels (Jackson and McCandless, 1978). Serum was deproteinized with trichloroacetic acid, then 0.5% barium chloride in 0.01% agarose was added and the samples read at 500 nm using a microtitre plate reader (XReadPlus, Tecan). A calibration curve was prepared using potassium sulfate standards. Serum and urine sulfate levels were normalized to total protein levels, which were quantitated using a protein microassay kit (Bio-Rad).

2.3. RT-PCR

Total RNA was extracted from individual livers of 1 year old mice using standard procedures (TRIzol reagent; Invitrogen). For RT-PCR, total RNA (2 μg) was reverse transcribed by using Moloney murine leukemia virus reverse transcriptase (Invitrogen). Primers (Supplemental Table 2) were used to amplify *Sirt1* (550 bp), *Hdac3* (598 bp), *Trp53* (356 bp), *Cd55* (486 bp), *Cat* (741 bp), *Tert* (717 bp), *Igfbp2* (450 bp), *Ccng* (610 bp), *Igf1a* (339 bp), *Igf1b* (391 bp), *Insr* (601 bp)

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and *Slc13a5* (564 bp) cDNA fragments. Each cDNA was co-amplified with *GAPDH* (control; 983 bp) using cycle parameters: 94 °C for 1 min; followed by 25 cycles of 94 °C for 1 min, 55 °C (*Sirt1*, *Hdac3*, *Cat*), 60 °C (*Igf1a*, *Igfbp2*, *Insr*, *Ccng*), 63 °C (*Trp53*, *Slc13a5*), 65 °C (*Cd55*) or 66 °C (*Tert*) for 1 min, and 72 °C for 1 min. PCR products in the linear phase of amplification were analyzed by densitometry (Scion Image Beta 4.0.2), and normalized to *GAPDH* as described previously (Lee et al., 2006).

2.4. Catalase assays

Liver was excised, washed in distilled H_2O, and homogenized in 0.07 M phosphate buffer pH 7.2. Catalase activity was measured in samples as previously described (Aebi, 1984).

2.5. Histology

Livers were dissected into approximately 50 volumes of 10% buffered formalin and fixed for 3 days prior to paraffin embedding. Embedded tissue was sectioned, stained with Hematoxylin and Eosin, and examined by light microscopy. Sections were scored for the presence or absence of neoplastic growth.

2.6. Statistical analyses

The statistical significance of differences between $Nas1^{+/+}$ and $Nas1^{-/-}$ groups was assessed by an unpaired Student's *t*-test, with $P<0.05$ considered significant. Differences in survival were analysed by the log-rank (Mantel–Cox) test, with the significance level set at $P<0.05$.

3. Results and discussion

Median lifespan is increased by ≈25% in both male and female *Nas1*−/− mice when compared to male and female *Nas1*+/+ mice, respectively (Fig. 1). In *Nas1*+/− mice, only females showed an increased lifespan by ≈20%. The observed sex differences are likely due to the pro-aging (antioxidant) effects of oestrogens, being more abundant in females. Survival trends were similar in male and female wild type (*Nas1*+/+) mice (Fig. 1). Food intake in *Nas1*−/− and *Nas1*+/+ mice is identical (Dawson et al., 2006), suggesting that the increased longevity (Fig. 1) of *Nas1*−/− mice may not be due to caloric restriction. To determine the role of circulating SO_4^{2-} on survival rate, serum SO_4^{2-} levels were measured in 1 yr old mice when differences in survival were observed (Fig. 1). Serum SO_4^{2-} levels were reduced (by ≈84%) in 1 yr old *Nas1*−/− mice (0.16 ± 0.04 mM) when compared *Nas1*+/+ mice (0.98 ± 0.02 mM) (Fig. 2A), which is similar to the levels previously found in 2 and 4 month old *Nas1*−/− and *Nas1*+/+ mice (Dawson et al., 2003). These data suggest NaS1 is essential for maintaining high (≈1.0 mM) circulating SO_4^{2-} levels up to at least 1 year of age in mice and that *Nas1*−/− mice may live longer due to hyposulfatemia.

To determine the possible mechanism of increased longevity in *Nas1*−/− mice, we measured liver mRNA levels of 12 genes which had previously been associated with increased lifespan or decreased susceptibility to liver tumours. Our previous data showing reduced circulating IGF-1 levels (Dawson et al., 2003) and altered hepatic transcriptional profile in 2 month old *Nas1*−/− mice (Dawson et al., 2006), indicated that it may be through the growth hormone/insulin/IGF-I signalling pathway. Our data showed that mRNA levels of *Tert*, *Ccng1*, *Igfbp2*, *Igf1a*, *Igf1b*, *Insr* and *Slc13a5* were ($P>0.05$) unaltered (data not shown), whereas *Sirt1*, *Hdac3*, *Trp53*, *Cd55* and *Cat* were significantly increased in the livers of 1 year old *Nas1*−/− mice when compared to *Nas1*+/+ mice (Fig. 2B). The most up-regulated transcripts were sirtuin-1 (≈60% increase), catalase (≈48% increase), CD55 and Trp53 (≈36% increase), which provide metabolic benefits of

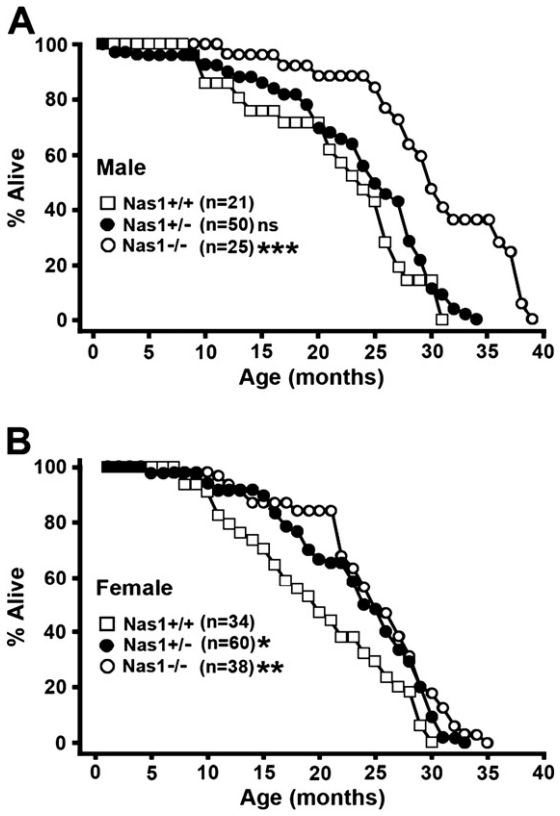

Fig. 1. Survival ages of individual (A) male and (B) female *Nas1*+/+, *Nas1*+/− and *Nas1*−/− mice. $^{*}P=0.0181$, $^{**}P=0.0055$, $^{***}P<0.0001$ and ns = non-significant (log-rank test) when compared to *Nas1*+/+ mice.

enhanced longevity in mice (Brown-Borg and Rakoczy, 2000; Leibiger and Berggren, 2006) and *C. elegans* (Braeckman and Vanfleteren, 2007). Despite *Cat* mRNA levels being significantly increased, catalase activity was unaltered in the livers of *Nas1*−/− mice (Fig. 2C), which agrees with previous data in mice (Brown-Borg and Rakoczy, 2000). Since NaS1 mRNA is not expressed in the liver (Beck and Markovich, 2000), the observed differential expression of *Sirt1*, *Hdac3*, *Trp53*, *Cd55* and *Cat* mRNA is due to reduced SO_4^{2-} delivery to *Nas1*−/− mouse livers (Lee et al., 2006).

The increased mRNA expression of *Trp53* (by ≈36%) and *Hdac3* (by ≈22%) (Fig. 2B), genes involved in tumour suppression (Chen et al., 1990; Mahlknecht et al., 1999), led us to compare the incidence of spontaneous hepatic neoplasms at 2 years of age, the age showing significant differences in survival rates between *Nas1*−/− and *Nas1*+/+ mice (Fig. 1A). Gross histological analysis revealed neoplasms in *Nas1*+/+ mice, but none in the livers of 2 year old *Nas1*−/− mice (Fig. 2D). These findings are comparable to hepatocarcinomas detected in 28–31 month old wild type mice (Walter et al., 2001). Our data suggest that *Nas1*−/− mice may be less susceptible to liver tumours, most likely due to reduced SO_4^{2-} delivery to the liver, by regulating the expression of genes associated with tumour suppression and increased longevity.

4. Conclusion

This is the first study to identify enhanced longevity and decreased liver tumours in a mouse model of hyposulfatemia. These findings are relevant to diet, hormones and analgesics which regulate blood SO_4^{2-} levels in mammals (Markovich, 2001) and prompts further assessment of ageing in humans with altered sulfate homeostasis.

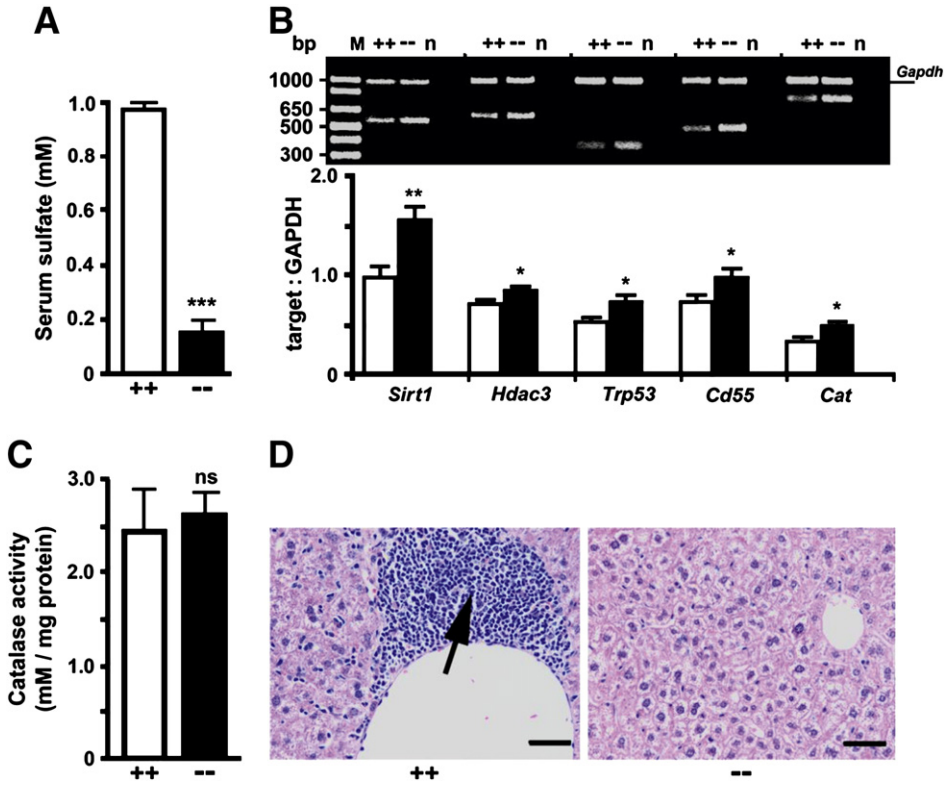

Fig. 2. Serum SO_4^{2-} levels and hepatic gene expression, catalase activity and histology in *Nas1*+/+ and *Nas1*−/− mice aged 1 year (A–C) and 2 years (D). (A) Decreased serum SO_4^{2-} levels in *Nas1*−/− mice. (B) RT-PCR products (top) showing a representative example of increased hepatic mRNA levels of *Sirt1*, *Hdac3*, *Trp53*, *Cd55* and *Cat* (bottom) in *Nas1*−/− mice. (C) Hepatic catalase activity in *Nas1*+/+ and *Nas1*−/− mice. (D) Representative photomicrographs of H&E-stained liver sections showing a neoplastic growth (*arrow*) found in 57% *Nas1*+/+ mice ($n=7$), but not in *Nas1*−/− mice ($n=7$). *Bar*, 50 μm. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.00001$ and ns = nonsignificant (unpaired Student *t* test) when compared to *Nas1*+/+ mice. Values represent mean ± SEM of 4–7 animals per group. Identical data was observed for males and females.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.exger.2011.05.008.

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