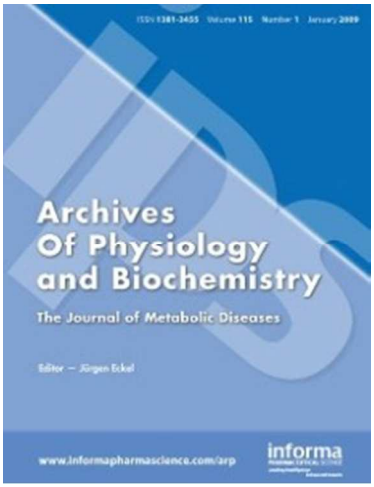

Age, weight and decompression sickness in rats

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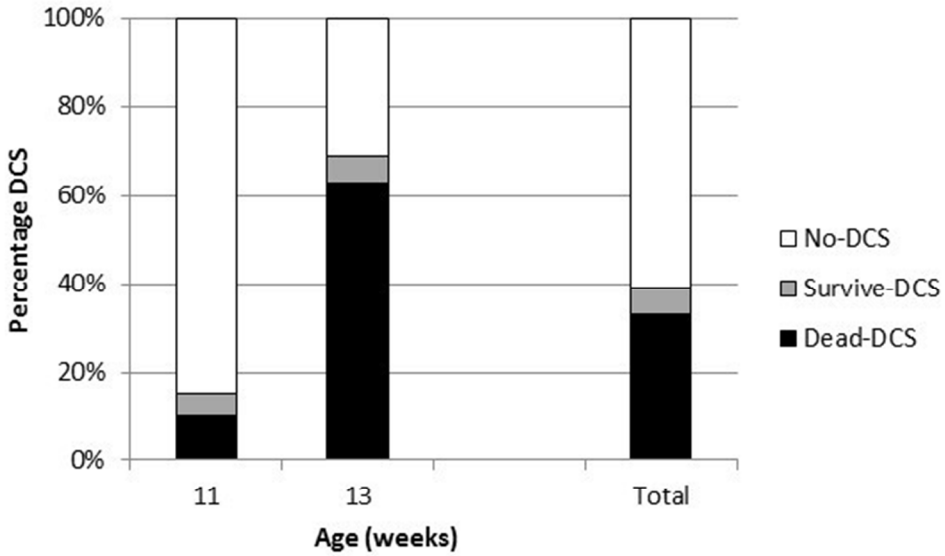

Decompression health outcome by age and overall
127x76mm (120 x 120 DPI)

Age, weight and decompression sickness in rats

by

Peter Buzzacott,* Michael Theron, Aleksandra Mazur, Qiong Wang, Kate Lambrechts, Ingrid Eftedal, Simin Berenji Ardestani, François Guerrero.

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Keywords: Decompression illness, diving, Long-Evans, regression modelling.

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Age, weight and decompression sickness in rats

Abstract

Objective: The aim of this study was to determine if, after controlling for weight, age is associated with decompression sickness (DCS) in rats.

Methods: Following compression-decompression, male rats aged 11 weeks were observed for DCS. After two weeks recovery surviving rats were re-dived using the same compression-decompression profile.

Results: In this experiment there was a clear difference between DCS outcome at ages 11 or 13 weeks in matched rats (p=0.002).

Discussion: Even with weight included in the model age was significantly associated with DCS (p=0.01), yet after removal of weight the association was much stronger (p=0.002).

Conclusion: We believe that age is likely to be found associated with the probability of DCS in a larger dataset with a wider range of parameters, after accounting for the effect of weight.

Introduction

Age is a factor of interest for decompression sickness (DCS) research in diving humans (Carturan et al., 2002, Carturan et al., 1999). Both diving and aging exert distinct influences over cardiovascular function though the nature of their relationship when combined remains uncertain (Boussuges et al., 2009). With senescence the cardiovascular system undergoes complex structural and functional changes (Ardestani et al., 2015

). Diving too may lead to vascular dysfunction and endothelial cell death (Lambrechts et al., 2013, Wang et al., 2014).

Aging induces phenotypical changes in human coronary arterioles that are similar to the age-associated remodeling of the walls of large arteries seen in rats, age is associated with increasing endothelial dysfunction in both humans and rodents (Lakatta, 2003) and age-related endothelial dysfunction has been observed in coronary arterioles of approximately 80-week old rats (Csiszar et al., 2002) corresponding to a human age of 90 years (Sengupta, 2013). Rats are also a useful model for researching DCS (Mazur et al., 2014), particularly as the majority of DCS research in humans is, by necessity, retrospective (Sulaiman et al., 1997). Since age-associated cardiovascular changes have been found comparable between humans and rats, we propose that rat model research might help elucidate the relationship between age and risk of DCS in humans. The number of data required for such an analysis is of the order of many hundreds therefore rat models offers a convenient alternative to studying DCS in humans. For rat research though, age is confounded by weight, which increases with age (Figure 1), and weight is a known risk factor for DCS in rats (Lillo and MacCallum, 1991, Arieli et al., 2007).

The aim of this study was to determine if age is associated with DCS outcome in rats after controlling for weight. The purpose of this study was support investment into a substantial modelling project to estimate the size of the effect of age upon DCS outcome in rats. If no correlation between age and DCS was observed in this study then the modelling endeavour to estimate the effect size of age upon DCS would not proceed.

Methods

Male Long-Evans rats (n=20) were obtained in two batches, two weeks apart, from Janvier SAS (Le Genest St Isle, France) at age 10 weeks. Long Evans were included in this experiment for their wide variation in weight at 11 weeks and their rapid growth (a median increase of 20% bodyweight) over the following two weeks (median 58g, range 20-90g). The rats were housed for one week before the experiment in the Faculty of Sciences and Techniques vivarium in standard conditions, (mean temperature 21.2°C +/- 0.2, relative humidity 27% +/- 16%, 12 hour light:dark cycle, 7am-7pm), during which they had access to water and rat chow *ad libitum*. Hydration was withdrawn 30 mins before compression. The rats were weighed on the day of diving before being compressed in a 170-litre hyperbaric chamber (Comex, Marseille, France). All dives commenced in the morning after 8am.

The air inside the chamber was compressed to 1000kPa at the rate of 100kPa per minute. Maximum pressure was held for 45-minutes followed by decompression at 100kPa per minute to 200kPa. Decompression was thereafter staged with five mins at 200kPa, five mins at 160kPa and 10 mins at 130kPa. This protocol has been shown to produce DCS signs in a predictable proportion of male rats aged 10-11 weeks (Buzzacott et al., 2014).

Following decompression the rats were swiftly removed from the chamber and observed for signs of DCS for one hour. DCS classification was No observable DCS (no-DCS)=0, respiratory distress or paralysis (survived-DCS)=1, or death within one hour (dead-DCS)=2. The diagnosis was noted by two observers in each case. The observation period ended at 60 minutes and mortality, morbidity or apparent health was noted.

After two weeks recovery the surviving rats were re-dived using the same compression-decompression profile. In rats at this post-adolescent stage of their lives, two weeks equates to a little over one year of young adult development in humans. This research, including

death as an endpoint, was approved by Universite de Bretagne Occidentale animal research ethic committee and the French Ministry of Agriculture (R-2011-FG-01). A pain-display scale was also approved by the ethic committee and any animal displaying signs at the pre-determined threshold (n=1) was immediately euthanized with an intraperitoneal injection of a lethal dose of sodium-pentobarbital. All rats showing signs of DCS below the threshold recovered within 30 mins and were indistinguishable from the other survivors at 60 mins, as has been previously reported (Lillo, 1988, Sallee and Adams, 1970).

ANALYSIS

Potential predictors of DCS outcome were analysed using SAS ver 9.3 (SAS, Cary, North Carolina). Significance of age was assessed using ordinal logistic regression with weight on the day of diving included in the model, (Eq. 1), since weight is known to have a significant effect upon likelihood of DCS (Mazur et al., 2014). The rats were compressed one or two at a time, in with other rats of different strains for other experiments to be reported elsewhere. Model fit of the dataset was optimised using the likelihood ratio test with one degree of freedom comparing the log likelihood (-2LL) of the full model (Eq. 1) with the diminished model, which is appropriate for small datasets when only one of two parameters is to be removed.

$$Ln\left(\frac{P_j}{1-P_j}\right) = \alpha_j + \beta_1 Age + \beta_2 Weight \quad (1)$$

The modelled probability (P) of a ternary DCS outcome state *j*, (of no-DCS, survived-DCS or dead-DCS), is been described in detail elsewhere (Buzzacott et al., 2014). Briefly, the probability of no-DCS (vs. DCS) is calculated, then the probability of dead-DCS (vs. alive). The probability of survived-DCS is then determined by subtracting the probabilities of no-DCS and dead-DCS from 1.0, which is the sum of all possible probabilities. Significance

was accepted at $p<0.05$ and we had 80% power to detect a difference of ±30% DCS within 20 rats at the $p<0.05$ level of significance.

Lastly, we predicted what theoretical effect the four rats who made only the first dive would have had if they had had the opportunity to live through the second dive, which would have moved the data towards the null hypothesis (that additional age had the opposite effect upon DCS outcome).

Results

Mean weight by age and block is shown in Table 1. The mean weight of Batch 1 at age 13 weeks effectively cancelled out the effect of weight in Batch 2 at age 11 weeks (Table 1, in bold). During the first dive one rat from each Batch died from DCS, one rat from Batch 1 was excluded from the second dive after the cage it was in was mislabelled and one rat from Batch 2 was euthanized after the first dive to alleviate pain. DCS outcome by age is shown in Figure 1.

The -2LL of the initial model was 46.967, the -2LL=54.458 after age was removed (difference=7.491) and the -2LL=47.439 after weight was removed (difference=0.472). Removing weight did not significantly worsen the model ($p>0.25$) but removing age did ($p<0.01$) therefore weight was removed from the model. Age was then shown to be significantly associated with the probability of suffering DCS ($p=0.002$). In this experiment there was a clear difference between DCS outcome at ages 11 or 13 weeks in matched rats. If the four rats excluded from the second dive had each hypothetically survived it then age would have remained significant ($p=0.008$).

Discussion

Even with weight included in the model age was significantly associated with DCS (p=0.01), yet after removal of weight the association was much stronger (p=0.002), therefore we believe that age is likely to be found associated with the probability of DCS in a large dataset with a wider range of parameters, after accounting for the effect of weight. The estimated size of the effect of age we observed (Fig. 1) was considerably greater than we previously reported in Sprague-Dawley rats between the ages of 11 and 13 weeks and therefore we speculate that the effect size in a larger analysis with multiple strains might be substantially smaller (Mazur et al., 2014). Lillo et al (2002) suggest weight has an exponential effect upon risk of DCS but this study did not primarily aim to estimate effect size, rather the aim was confirm if a relationship exists between age and DCS after controlling for weight. Given that the mean weight in Batch 1 at 13 weeks was equivalent to the mean weight in Batch 2 at 11 weeks, weight was controlled for through study design and the effect size of weight not explored. Given the outcome had three ordinal levels, ordinal logistic regression was an appropriate method of model-fitting in this instance.

There are many limitations in a small test-retest experiment such as this, including that the effect of a second exposure (through matched controls) is a potential confounder. The second dive was made after two weeks recovery and all rats appeared healthy but we cannot exclude the possible influence of stress, either more or less, than during the first exposure. It is also possible that the rats' resistance deteriorated over an additional two weeks of housing in our vivarium, for example if they were subjected to low temperature or humidity, noise, etc, but we consider this unlikely because other rats were housed in the same vivarium over the same period and their health showed no signs of housing stress, not to mention that the rats in this

experiment showed considerable weight gain over the two week rest period. Indeed, prior hyperbaric exposure has been shown to acclimate rats to compression/decompression, increasing their likelihood of survival (Montcalm-Smith et al., 2010).

Hormonal levels may have increased during the two week interlude but that might not confound the effect of age since hormonal levels are tied with age, especially when sexual maturity is first reached as in this study. Hormones were recently suggested as a potential explanation for the difference in DCS outcome between male and female rats (Mazur et al., 2014) but how exactly additional age effected susceptibility to DCS is not clear from this study. The first dive was made in the chamber with larger rats from other experiments but in the second dive the rats in this experiment unaccompanied in the chamber. Again, possibly stress confounded the difference, for example if pheromones were detected from other strains in the first experiment and this was somehow protective, (although there is nothing to suggest this possibility in the literature). Lastly, we cannot predict what effect the four rats who made only the first dive would have actually had if they had had the opportunity to make the second dive. We showed that, hypothetically, even if they had survived the second dive then age would have remained significant but we cannot know what their weights would have been at age 13 weeks and so this is tentative at best.

Conclusion

Rat models appear to have the potential to contribute to mankind's understanding of the influence age exerts upon risk of DCS among divers. Even given the above limitations we are now confident enough of the existence of an association to proceed with modelling the effect size of age upon DCS outcome in rats in a much larger study.

Declaration of Interests

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Table 1: Mean weight (SD) per batch by age

Batch		1		2	
Age	(weeks)	11 (n=10)	13 (n=8)	11 (n=10)	13 (n=8)
Weight	(g)(SD)	268 (12)	**358** (11)	**336** (12)	400 (21)

List of Figures

Age, weight and decompression sickness in rats

by

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Keywords: Decompression illness, diving, Long-Evans, regression modelling.

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Age, weight and decompression sickness in rats

Abstract

Objective: The aim of this study was to determine if, after controlling for weight, age is associated with decompression sickness (DCS) in rats.

Methods: Following compression-decompression, male rats aged 11 weeks were observed for DCS. After two weeks recovery surviving rats were re-dived using the same compression-decompression profile.

Results: In this experiment there was a clear difference between DCS outcome at ages 11 or 13 weeks in matched rats (p=0.002).

Discussion: Even with weight included in the model age was significantly associated with DCS (p=0.01), yet after removal of weight the association was much stronger (p=0.002).

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Introduction

Age is a factor of interest for decompression sickness (DCS) research in diving humans (Carturan et al., 2002, Carturan et al., 1999). Both diving and aging exert distinct influences over cardiovascular function though the nature of their relationship when combined remains uncertain (Boussuges et al., 2009). With senescence the cardiovascular system undergoes complex structural and functional changes ~~and risk of cardiovascular disease increases~~ (Ardestani et al., 2015

Jousilahti et al., 1999). In a French study of 200 professional divers (mean age 38 years), 2.5% had a high 10-year risk and 34% had an intermediate 10-year risk of an acute coronary event (Pougnet et al., 2012). Diving too may lead to vascular dysfunction and endothelial cell death (Lambrechts et al., 2013, Wang et al., 2014). Obad et al found that post-dive flow-mediated dilation of the brachial artery in divers remained reduced for two days after a single air dive (Obad et al., 2007). Right ventricular dysfunction and increased pulmonary artery pressure likewise took longer than 24 hours to reverse (Obad et al., 2007).

Aging induces phenotypical changes in human coronary arterioles that are similar to the age-associated remodeling of the walls of large arteries seen in rats, age is associated with increasing endothelial dysfunction in both humans and rodents (Lakatta, 2003) and age-related endothelial dysfunction has been observed in coronary arterioles of approximately 80-week old rats (Csiszar et al., 2002) corresponding to a human age of 90 years (Sengupta, 2013). Rats are also a useful model for researching DCS (Mazur et al., 2014), particularly as the majority of DCS research in humans is, by necessity, retrospective (Sulaiman et al., 1997). Since age-associated cardiovascular changes have been found comparable between humans and rats, we propose that rat model research might help elucidate the relationship between age and risk of DCS in humans. The number of data required for such an analysis is of the order of many hundreds therefore rat models offers a convenient alternative to studying DCS in humans. For rat research though, age is confounded by weight, which increases with age (Figure 1), and weight is a known risk factor for DCS in rats (Lillo and MacCallum, 1991, Arieli et al., 2007). Sex has also recently been shown associated with the risk of DCS in rats and VGE grades have been found higher in male divers over females (Mazur et al., 2014, Buzzacott et al., 2014, Boussuges et al., 2009). By age 55 years the lifetime risks of

~~cardiovascular disease in humans are similar in males and females although first manifestations differ considerably (Leening et al., 2014).~~

The aim of this study was to determine if~~, after controlling for weight,~~ age is associated with DCS outcome in rats after controlling for weight. The purpose of this study was ~~to assess the viability of~~support investment into a substantial modelling project to estimate the size of the effect ~~size~~ of age upon DCS outcome in rats. If no ~~significant raw effect~~ correlation between age and DCS was observed in this study then the modelling endeavour to estimate the effect size of age upon DCS would not proceed.

Methods

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ANALYSIS

Potential predictors of DCS outcome were analysed using SAS ver 9.3 (SAS, Cary, North Carolina). Significance of age was assessed using ordinal logistic regression with weight on the day of diving included in the model, (Eq. 1), since weight is known to have a significant effect upon likelihood of DCS (Mazur et al., 2014). The rats were compressed one or two at a

time, in with other rats of different strains for other experiments to be reported elsewhere. Model fit of the dataset was optimised using the likelihood ratio test with one degree of freedom comparing the log likelihood (-2LL) of the full model (Eq. 1) with the diminished model, which is appropriate for small datasets when only one of two parameters is to be removed.

$$Ln\left(\frac{P_j}{1-P_j}\right) = \alpha_j + \beta_1 Age + \beta_2 Weight \quad (1)$$

The modelled probability (P) of a ternary DCS outcome state *j*, (of no-DCS, survived-DCS or dead-DCS), is been described in detail elsewhere (Buzzacott et al., 2014). Briefly, the probability of no-DCS (vs. DCS) is calculated, then the probability of dead-DCS (vs. alive). The probability of survived-DCS is then determined by subtracting the probabilities of no-DCS and dead-DCS from 1.0, which is the sum of all possible probabilities. Significance was accepted at $p<0.05$. ~~W~~ and we had 80% power to detect a difference of ±30% DCS within 20 rats at the $p<0.05$ level of significance.

Lastly, we predicted what theoretical effect the four rats who made only the first dive would have had if they had had the opportunity to live through the second dive, which would have moved the data towards the null hypothesis (that additional age had the opposite effect upon DCS outcome).

Results

Mean weight by age and block is shown in Table 1. The mean weight of ~~Block~~ Batch 1 at age 13 weeks effectively cancelled out the effect of weight in ~~Block~~ Batch 2 at age 11 weeks (Table 1, in bold). During the first dive one rat from each ~~Block~~ Batch died from DCS, one

rat from ~~Block~~ Batch 1 was excluded from the second dive after the cage it was in was mislabelled and one rat from ~~Block~~ Batch 2 was euthanized after the first dive to alleviate pain. DCS outcome by age is shown in Figure 1~~2~~.

The -2LL of the initial model was 46.967, the -2LL=54.458 after age was removed (difference=7.491) and the -2LL=47.439 after weight was removed (difference=0.472). Removing weight did not significantly worsen the model ($p>0.25$) but removing age did ($p<0.01$) therefore weight was removed from the model. Age was then shown to be significantly associated with the probability of suffering DCS ($p=0.002$). In this experiment there was a clear difference between DCS outcome at ages 11 or 13 weeks in matched rats. If the four rats excluded from the second dive had each hypothetically survived it then age would have remained significant ($p=0.008$).

Discussion

Even with weight included in the model age was significantly associated with DCS ($p=0.01$), yet after removal of weight the association was much stronger ($p=0.002$), therefore we believe that age is likely to be found associated with the probability of DCS in a large dataset with a wider range of parameters, after accounting for the effect of weight. The estimated size of the effect of age we observed (Fig. 1~~2~~) was considerably greater than we previously reported in Sprague-Dawley rats between the ages of 11 and 13 weeks and therefore we speculate that the effect size in a larger analysis with multiple strains might be substantially smaller (Mazur et al., 2014). Lillo et al (2002) suggest weight has an exponential effect upon risk of DCS but this study did not primarily aim to estimate effect size, rather the aim was

confirm if a relationship exists between age and DCS after controlling for weight. Given that the mean weight in Batch 1 at 13 weeks was equivalent to the mean weight in Batch 2 at 11 weeks, weight was controlled for through study design and the effect size of weight not explored. Given the outcome had three ordinal levels, ordinal logistic regression was an appropriate method of model-fitting in this instance. ~~Long-Evans were included in this experiment for their wide variation in weight at 11 weeks and their rapid growth (a median increase of 20% bodyweight) over the following two weeks (median 58g, range 20-90g). This may well differ to the characteristics of the dataset we will use to determine the effect size of weight upon DCS outcome and in this respect this experiment should be considered 'proof of concept' rather than an assessment of effect size. Strain has recently been found not associated with DCS outcome in three rat studies and, though less common than Sprague-Dawley or Wistar, Long-Evans have previously been used in hyperbaric research (Buzzacott et al., 2014, Mazur et al., 2014, Torbati et al., 1995).~~

There are many limitations in a small test-retest experiment such as this, including that the effect of a second exposure (through matched controls) is a potential confounder. The second dive was made after two weeks recovery and all rats appeared healthy but we cannot exclude the possible influence of stress, either more or less, than during the first exposure. It is also possible that the rats' resistance deteriorated over an additional two weeks of housing in our vivarium, for example if they were subjected to low temperature or humidity, noise, etc, but we consider this unlikely because other rats were housed in the same vivarium over the same period and their health showed no signs of housing stress, not to mention that the rats in this experiment showed considerable weight gain over the two week rest period. Indeed, prior hyperbaric exposure has been shown to acclimate rats to compression/decompression, increasing their likelihood of survival (Montcalm-Smith et al., 2010).

Hormonal levels may have increased during the two week interlude but that might not confound the effect of age since hormonal levels are tied with age, especially when sexual maturity is first reached as in this study. Hormones were recently suggested as a potential explanation for the difference in DCS outcome between male and female rats (Mazur et al., 2014) – but how exactly additional age effected susceptibility to DCS is not clear from this study. The first dive was made in the chamber with larger rats from other experiments but in the second dive the rats in this experiment unaccompanied in the chamber. Again, possibly stress confounded the difference, for example if pheromones were detected from other strains in the first experiment and this was somehow protective, (although there is nothing to suggest this possibility in the literature). Lastly, we cannot predict what effect the four rats who made only the first dive would have actually had if they had had the opportunity to make the second dive. We showed that, hypothetically, even if they had survived the second dive then age would have remained significant but we cannot know what their weights would have been at age 13 weeks and so this is tentative at best.

Conclusion

Rat models appear to have the potential to contribute to mankind's understanding of the influence age exerts upon risk of DCS among divers. Even given the above limitations we are now confident enough of the existence of an association to proceed with modelling the effect size of age upon DCS outcome in rats in a much larger study.

Declaration of Interests

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Table 1: Mean weight (SD) per ~~block~~ batch by age

Batch~~lock~~		1		2	
Age	(weeks)	11 (n=10)	13 (n=8)	11 (n=10)	13 (n=8)
Weight	(g)(SD)	268 (12)	**358** (11)	**336** (12)	400 (21)

List of Figures