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RESEARCH ARTICLE

Glucose tolerance predicts survival in old zebra finches

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ABSTRACT

The capacity to deal with external and internal challenges is thought to affect fitness, and the age-linked impairment of this capacity defines the ageing process. Using a recently developed intra-peritoneal glucose tolerance test (GTT), we tested for a link between the capacity to regulate glucose levels and survival in zebra finches. We also investigated for the effects of ambient factors, age, sex, and manipulated developmental and adult conditions (i.e. natal brood size and foraging cost, in a full factorial design) on glucose tolerance. Glucose tolerance was quantified using the incremental 'area under the curve' (AUC), with lower values indicating higher tolerance. Glucose tolerance predicted survival probability in old birds, above the median age, with individuals with higher glucose tolerance showing better survival than individuals with low or intermediate glucose tolerance. In young birds there was no association between glucose tolerance and survival. Experimentally induced adverse developmental conditions did not affect glucose tolerance, but low ambient temperature at sampling and hard foraging conditions during adulthood induced a fast return to baseline levels (i.e. high glucose tolerance). These findings can be interpreted as an efficient return to baseline glucose levels when energy requirements are high, with glucose presumably being used for energy metabolism or storage. Glucose tolerance was independent of sex. Our main finding that old birds with higher glucose tolerance had better survival supports the hypothesis that the capacity to efficiently cope with a physiological challenge predicts lifespan, at least in old birds.

KEY WORDS: Early-life environment, Glucose regulation, Glucose tolerance test, Survival, Age-dependent mortality, *Taeniopygia guttata*

INTRODUCTION

Internal and external perturbations such as disease, adverse weather conditions and low food availability challenge the physiological homeostasis of organisms, and a range of mechanisms has evolved to protect this internal environment from such challenges (López-Maury et al., 2008; McEwen and Wingfield, 2003). These homeostatic mechanisms allow organisms to maintain physiological parameters within a life-sustaining range under changing conditions (McEwen and Wingfield, 2003; Romero et al., 2009), and the robustness of such mechanisms is therefore expected to be favored by selection (Nijhout et al., 2017). During early life, a failure in one of the processes involved in homeostasis is more likely to be buffered by other mechanisms, but with age, a decline in the capacity to cope with, or recover from, adversity occurs (Boonekamp et al., 2015; Cohen et al., 2020; Ukraintseva et al., 2021), thereby increasing mortality. Indeed, Medawar (1952) defined senescence as 'that change of the body functions and sensibilities and energies which accompanies ageing, and which renders the individual progressively more likely to die from accidental causes of random incidence'. From a population perspective, the deviation from the population mean (i.e. population mean interpreted as state of least dysregulation) of physiological markers has been proposed as a signal of physiological dysregulation and has been shown to increase with age in humans and wild birds (Milot et al., 2014; Cohen et al., 2014). However, whether this deviation from the population mean mirrors an individual's reduced capacity to maintain homeostatic regulation increasing vulnerability (*sensu* Varadhan et al., 2008) or merely reflects different optimal levels remains to be established.

Coping with external challenges is often energetically demanding (e.g. Pendlebury et al., 2004; Fletcher et al., 2012; Jimeno et al., 2018b; Carneiro-Nascimento et al., 2020). Therefore, physiological processes involving energy metabolism are of special interest to understand how organisms deal with challenging conditions. In vertebrates, glucose is one of the main sources of metabolizable energy (Bernard et al., 2003; Braun and Sweazea, 2008; Weber, 2011), and glucose regulation maintains circulating glucose levels within a constant range despite the continuous intake by the individual and uptake by specific body tissues. Variations in this regulatory capacity depend on the proper sensing of circulating glucose levels and the correct release and action of hormones required for clearance and inhibition of the endogenous production (Braun and Sweazea, 2008; Polakof et al., 2011). Impaired glucose regulation has been linked to health problems and ageing in humans and laboratory models (Semba et al., 2010; Picard et al., 2014; Regan et al., 2020). Hence, this regulatory capacity has been used as a measure of biological resilience in human research (Ukraintseva et al., 2021) and may be considered a biomarker of individual condition, in the sense that it provides information about the performance of vital processes (Hill, 2011). Moreover, glucose regulation has been shown to be repeatable within individuals through time (Pratt et al., 2005; Bröjer et al., 2013; Montoya et al., 2018, 2020), suggesting that this trait can be a potential target of selection.

Biomedical research suggests a link between glucose regulation and lifespan (e.g. Zang et al., 2020), but we are not aware of studies exploring this association in a more ecological context, even though associations between physiological status and lifespan can be very different in (semi-) ecological contexts from that in humans or in a laboratory context (Briga and Verhulst, 2015). However, recent pioneering studies have shown that chronic social stress (Carneiro-

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Nascimento et al., 2020) and viral infections (McGraw et al., 2020) affect glucose levels, suggesting a reduction of the capacity to maintain glucose homeostasis (but see Vera et al., 2008). Birds are interesting models with which to study glucose regulation, because they typically have glucose levels that would have detrimental consequences on mammals of similar body mass (Holmes et al., 2001; Scanes and Braun, 2013).

Here, using zebra finches (*Taeniopygia guttata*), we followed a stimulus–response approach to challenge glucose regulation (Varadhan et al., 2008). This approach was used aiming to evaluate whether variation in glucose regulatory capacity predicts survival of individuals exposed to challenging ecological conditions during development (i.e. growing up in a large brood) and adulthood (i.e. high foraging costs). These experimentally induced challenging conditions have previously been associated with smaller size at adulthood and lower survival probability in the same population (Briga et al., 2017). Accordingly, if effects of glucose regulation capacity contributed to the experimental and age influence on survival, then we expect that: (i) birds exposed to hard conditions during development and/or adulthood will have lower capacity to regulate glucose levels, (ii) the capacity to regulate glucose will decline with age, and (iii) individuals with lower capacity to regulate glucose levels will have reduced survival, compared with individuals with higher capacity. Considering that the ability to regulate glucose may fail resulting in either too low (hypoglycemia) or too high glucose levels (hyperglycemia), we also contemplate the possibility that individuals with intermediate levels of glucose regulation will have higher survival probability when compared with individuals with lower and higher levels (Kowall et al., 2011). Likewise, we anticipated that the association between glucose regulation capacity and survival may depend on age, with young birds being less sensitive to impaired glucose regulatory capacity than old birds.

MATERIALS AND METHODS

Birds and housing

One hundred twenty-two adult zebra finches, 60 males and 62 females with an average age of 3.52±0.18 years (range 0.97–8.64 years), were randomly selected from the birds in an ongoing long-term experiment (see below) at the University of Groningen, The Netherlands (53°13′0″N, 6°33′0″E). Birds were bred indoors (see below), and after 120 days of age and through this study, were housed in single-sex outdoor aviaries (310×210×150 cm, L×H×W) with *ad libitum* access to tropical seed mixture, cuttlebone, water and sand (but see experimental treatments). In addition, birds were supplemented three times per week with 0.42 g (per bird) of a commercial nutritional supplement (Bogena, Hedel, The Netherlands).

Experimental treatments

Developmental conditions

All birds included in the study were cross-fostered as chicks to either a small (2 chicks) or a large brood (6 chicks) when the oldest chick of the birth nest was 4 to 5 days old. Siblings were assigned to different foster nests and stayed with their foster parents until nutritional independence (35 days old). Brood sizes were within the range observed in wild zebra finches (Zann, 1996). Between ages 35 and ∼120 days, birds were housed in indoor cages (153×76×110 cm, L×W×H) together with up to 40 other same-sex young and four adults (2 males and 2 females).

Adult foraging conditions

When approximately 120 days old, birds were moved to single-sex outdoor aviaries. Four aviaries (2 male and 2 female aviaries) were assigned to the benign foraging condition and four (2 male and 2 female aviaries) to the hard foraging condition. Each aviary contained 15–25 birds. Food was supplied in a food container (120×10×60 cm, L×W×H) suspended from the ceiling with 10 holes in the sides. In the benign foraging condition, there were perches beneath the holes to allow the birds to eat while perched. In the hard foraging condition, perches were removed from the food containers; thus birds had to fly from a distant perch to obtain the seeds and fly back to the perch to eat them (for details, see Koetsier and Verhulst, 2011).

All birds had their body mass measured monthly (Briga et al., 2019). In the present study, we used the body mass measurement closest in time after the glucose tolerance test (mean=2.9 days, s.d.=1.1 days). By the beginning of the measurements, the youngest bird sampled was 11.6 months old, and therefore it had been under the adult foraging conditions for 229 days. During the time on this study, individuals underwent physiological measurements of plasma corticosterone and metabolic rate and two blood sampling sessions per year for telomere length measurement.

Intra-peritoneal glucose tolerance test

We performed an intra-peritoneal glucose tolerance test (IP-GTT) between 24 September and 3 November 2014 as described in Montoya et al. (2020). Briefly, birds were taken from their aviaries and kept in a small box (40×40×15 cm, L×W×H) in a room with dim light together with two other birds, each in its own box, without access to food or water for 30 min, to ensure glucose levels were not associated with recent food consumption. Birds were maintained in these same conditions before and throughout the IP-GTT. Glucose injection increases blood glucose levels above to what has been reported in response to the handling protocol required for performing the IP-GTT (Montoya et al., 2020). During the IP-GTT, four 70 µl blood samples were taken from the brachial vein and collected in heparinized capillaries. Immediately after taking the first sample, we injected intraperitoneally 100 µl of 30% glucose [D-(+)-glucose C6H12O6, Sigma-Aldrich, Steinheim, Germany] diluted in saline solution, and blood samples were taken at 10, 20 and 40 min after glucose injection (following Montoya et al., 2020). After completing the IP-GTT, birds were individually maintained (in separated boxes) in a warm room with *ad libitum* access to food and water for 30 min before being reintroduced into their aviaries. The date, hour and temperature at the start of the IP-GTT were recorded for each individual. Temperature information was obtained from https://www.timeanddate.com/sun/netherlands/groningen. All procedures were evaluated and approved by the Animal Experimentation Committee of the University of Groningen (license number 5150).

After collection, blood samples were immediately diluted in a heparin (500 IU ml^{-1}) 0.01% EDTA solution (first blood sample: 20× diluted; post-injection samples: 30× diluted). After dilution, samples were first stored on ice and then frozen at −20°C, for up to 48 h until measurement. Glucose levels in whole blood were measured in duplicate using Hoffman's ferricyanide method in a Technicon autoanalyzer (Beckman Coulter LX20PRO). Repeatability calculated over the duplicates was 0.78 (n=538 samples, 95% CI: 0.74–0.80), and hence the 'extrapolated repeatability' of the measurements was 0.88 (Nakagawa and Schielzeth, 2010).

Glucose tolerance is defined as the ability to dispel a glucose load and was estimated as the incremental area under the glucose curve (AUC). AUC was calculated from the glucose levels measured in the four blood samples taken during the IP-GTT, as described in

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Montoya et al. (2020). In brief, we calculated the incremental AUC using the trapezoidal method (Tai, 1994), by adding the adjacent areas resulting from successive glucose measurements 0 (i.e. immediately before, G0), 10 (G10), 20 (G20) and 40 min (G40) after the intra-peritoneal glucose injection. Initial glucose levels (G0) were subtracted from all the subsequent measurements before calculating AUC. Therefore, a lower AUC after an external glucose load was considered as a stronger response as it indicates a faster return to baseline levels. However, given an AUC, the curve may have different shapes. To obtain more detailed information on the regulatory capacity, variation in curve shape can be quantified by decomposing the response in robustness, defined as the magnitude of the deviation from initial levels (G0) reached at the highest level achieved during the GTT (higher levels reflecting lower robustness), and resilience, defined as completeness of the recovery reached at the end (G40) of the trial with respect to initial levels (G0) (higher levels reflecting lower resilience) (Ukraintseva et al., 2016). Therefore, as a follow-up to the analysis of the AUC results, we tested whether observed patterns could be attributed to changes in robustness, resilience or both.

Statistical analyses

To evaluate the effects of environmental variables, experimental treatments and the individual's factors on GTT performance, linear mixed models were fitted using lme4 in R software version 4.0.0 (Bates et al., 2015; https://www.r-project.org/). Continuous variables, including glucose tolerance measured as AUC, were standardized (i.e. subtracting the mean and dividing by the standard deviation). We analyzed the association between glucose tolerance and survival with Cox proportional hazards (CPH) models (Therneau and Grambsch, 2000) using the function 'coxme' of the 'coxme' package (https://cran.r-project.org/package=coxme). The CPH model is essentially a regression model in which the hazard, i.e. the probability of dying at time t, is a function of a number of predictor variables, with their coefficients capturing the predictor's exponential increase in mortality per time unit relative to an empirically estimated baseline. CPH models can have different time scales (e.g. age versus time-on-study, with 0 starting at birth versus sampling age). Here, we followed the guidelines developed by Kom et al. (1997) and by Liestøl and Andersen (2002), which showed that when the predictor variable is a state variable without a specific time of onset, it is better to use age as a time scale (Kom et al., 1997; Liestøl and Andersen, 2002, p. 3709). Our experimental design has a staggered or delayed entry, and hence is subject to left truncation (Commenges et al., 1998; Therneau and Grambsch, 2000; Pencina et al., 2007). We account for this in two ways. First, we included the age at measurement as a predictor variable, which also allows us to test for age-specific effects. Note that age at measurement fulfilled the proportionality assumption, as tested with the function 'cox.zph' (χ^2=0.12, P=0.72). Second, we included left truncation following the 'interval censoring' approach as described by Therneau and Grambsch (2000).

The sample size was 122 individuals, of which 111 had died by 2 February 2019. The remaining 11 individuals were either alive at the end of the experiment or died from accidental causes (Briga et al., 2019), and therefore were included as censored in the analyses. During the time elapsed between the GTT and the cut-off point for survival analyses, individuals underwent other physiological measurements such as plasma corticosterone levels, metabolic rate and telomere length. In all analyses, we corrected for potential pseudoreplication owing to the birds' joint housing by including aviary as a random intercept. Females suffer a higher mortality rate than males, and this difference increases with age (Briga et al., 2017, 2019); therefore, we included sex in the models (as a strata, resulting in separate baseline hazard functions fitted for each sex). We tested for age-specific effects of our predictor variables by including their interaction with sampling age (continuous variable) (Therneau et al., 2019). Finally, considering that glucose tolerance may affect survival in a non-linear way and that this effect may depend on the individual's age, we also included in the models the interaction between glucose tolerance estimated as AUC, and AUC squared, with the sampling age (Kowall et al., 2011). We checked the CPH proportionality assumption using the function 'cox.zph' of the package 'survival' (https://cran.r-project.org/package=survival) and the Schoenfeld, beta, deviance and Martingale residuals with the package 'survminer' (https://CRAN.R-project.org/package=survminer), and found no evidence of violation of the assumptions.

RESULTS

Glucose tolerance, ambient/individual factors and experimental treatments

The AUC was used to integrate glucose levels over the IP-GTT, with high levels of AUC indicating low tolerance. AUC was high at warm ambient temperatures (Table 1, Fig. 1) and low in individuals with high body mass (Table 1) and in birds living in the hard foraging environment (Table 1, Fig. 2). Interestingly, AUC was low in the youngest and oldest individuals (Table 1, Fig. 3). No variation in AUC was explained by rearing brood size, its interaction with the foraging treatment, the individual's sex, or the day length or sampling hour (Table 1).

The robustness of the response to a glucose challenge was estimated as the magnitude of the deviation from baseline levels (G0), reached at the highest point of the GTT, with higher deviation reflecting lower robustness. Similar to AUC, robustness was lower at warm temperatures (Table 1), and higher in individuals with high body mass (Table 1) and those sampled at young and old ages (Table 1). Experimental treatments (brood size and foraging effort), their interaction, sex, sampling hour and day length were not significantly associated with robustness (Table 1). The resilience of the response to a glucose challenge was estimated as the completeness of the recovery (relative to the initial levels, G0) reached 40 min after the glucose injection (G40), with higher values reflecting poorer resilience. Resilience was associated with ambient temperature and day length, with individuals achieving poorer recovery at warmer temperatures and during shorter days (Table 1), but other variables were not significantly related to resilience (Table 1). Robustness and resilience were both strongly correlated with the AUC (robustness: r=0.92, $F_{1,118.97}$=281.736, P<0.0001; resilience: r=0.75, $F_{1,118.67}$=14.489, P<0.001). Adding baseline glucose to the models in Table 1 did not qualitatively change the results, and baseline glucose did not predict the AUC ($F_{1,110}$=0.24, P=0.62), robustness ($F_{1,109.98}$=1.65, P=0.20) or resilience ($F_{1,109.08}$=0.36, P=0.55).

Glucose tolerance and survival

When testing for associations between the AUC and survival, we considered linear as well as non-linear effects. Indeed, we found AUC levels to have a non-linear, age-dependent (fitted as a continuous variable) effect on survival (Table 2, Fig. 4), a result confirmed when correcting for left truncation following Therneau and Grambsch (2000) (Table S1). To better understand the interaction between the AUC squared and sampling age on survival, we categorized sampling age using the higher limit in

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Table 1. General linear mixed models exploring the associations of glucose tolerance measured as area under the glucose curve (AUC), robustness and resilience with ambient variation (temperature, day length and sampling about), individual factors (body mass, sex and sampling age) and experimental treatments (brood size and foraging effort)

	AUC				Robustness				Resilience			
Fixed effects	Coef.±s.e.	d.f.	*F*	*P*	Coef.±s.e.	d.f.	*F*	*P*	Coef.±s.e.	d.f.	*F*	*P*
Intercept	1.61±0.96	111	–	–	1.40±0.90	110.45	–	–	1.36±0.95	110.49	–	–
Temperature	**0.36±0.10**	**111**	**12.99**	**<0.001**	**0.38±0.10**	**110.99**	**14.15**	**<0.001**	**0.26±0.11**	**109.64**	**5.91**	**0.02**
Day length	−0.17±0.10	111	2.69	0.10	−0.13±0.10	109.04	1.58	0.21	**−0.23±0.11**	**109.77**	**4.42**	**0.04**
Hour	−0.01±0.10	111	0.01	0.92	−0.05±0.10	110.48	0.31	0.58	0.09±0.10	106.51	0.79	0.38
Body mass	**−0.21±0.08**	**111**	**6.34**	**0.01**	**−0.12±0.06**	**109.92**	**5.06**	**0.03**	−0.08±0.06	110.65	1.93	1.17
Sex (males)	0.21±0.17	111	1.59	0.21	0.32±0.21	5.903	2.42	0.17	0.26±0.18	5.391	2.05	0.21
Sample age	0.32±0.17	111	3.54	0.06	**0.39±0.17**	**109.12**	**5.13**	**0.02**	0.009±0.18	109.86	0.002	0.96
Sample age squared	**−0.04±0.02**	**111**	**4.60**	**0.03**	**−0.05±0.02**	**109.05**	**6.71**	**0.01**	−0.005±0.02	109.79	0.06	0.81
Foraging treatment	**−0.42±0.17**	**111**	**5.83**	**0.02**	−0.36±0.27	6.402	3.46	0.11	−0.35±0.25	5.99	2.59	0.16
Brood size	−0.09±0.17	111	0.28	0.60	−0.04±0.24	109.45	0.23	0.63	−0.10±0.25	109.88	0.10	0.75
Brood size×Foraging treatment	−0.07±0.33	111	0.05	0.82	−0.08±0.33	110.04	0.06	0.81	0.09±0.35	110.53	0.07	0.79
Random effects	Variance				Variance				Variance			
Aviary	<0.001				0.032				0.007			
Residual	0.775				0.757				0.860			

Note that all continuous variables were transformed to a standard normal distribution prior to analyses.
Table shows the coefficients (±s.e.) and denominator degrees of freedom (d.f.) of variables in the initial model; no model reduction was performed. In bold are fixed variables that explain variation of the glucose tolerance metric of interest (i.e. AUC, robustness or resilience).

our detection range, 4 years, as the cut-off, but note that any cut-off between 1 and 4 years gave consistent conclusions. This analysis confirmed that the quadratic effect of AUC on survival was present in old birds only [quadratic term for >4 years: exp(coef)=0.695 ±0.169, z=−2.15, P=0.032; quadratic term for <4 years; exp(coef)=1.039±0.117, z=0.33, P=0.74]. Old birds with intermediate and high AUC levels (i.e. low glucose tolerance) had lower predicted survival than old birds with low AUC values; in young birds there was no association between AUC levels and predicted survival (Fig. 4B, relative hazards were obtained

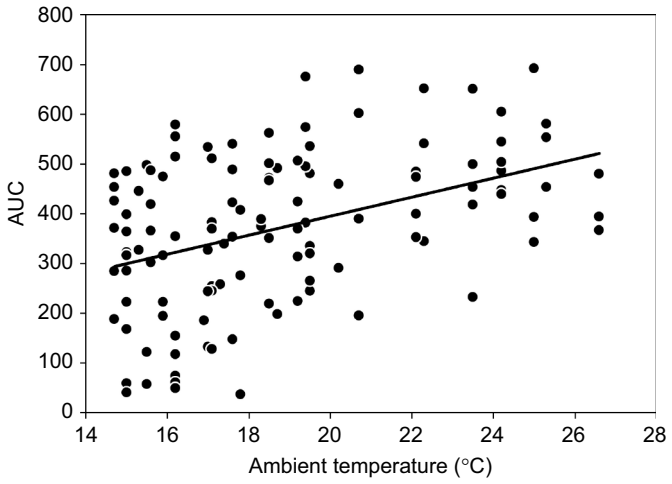

Fig. 1. Birds had higher area under the glucose curve (AUC; mmol l^{-1}) values after a glucose intra-peritoneal injection at more elevated ambient temperatures, indicating lower glucose tolerance at warmer temperatures (LMM, $F_{1,111}$=12.99, P<0.001). Glucose tolerance was measured as incremental area under the glucose curve (AUC) after an exogenous glucose challenge; adjacent areas resulting from four glucose measurements were added after subtracting baseline glucose levels. Higher AUC values represent lower glucose tolerance, whereas lower AUC values correspond to higher glucose tolerance. Values of ambient temperature were retrieved from https://www.timeanddate.com/sun/netherlands/groningen and correspond to the day when individuals were blood sampled. Glucose tolerance test was performed in 122 adult zebra finches.

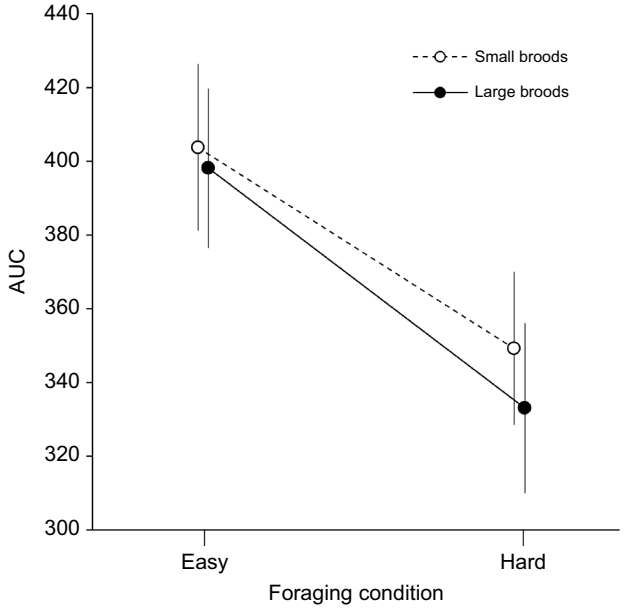

Fig. 2. Birds exposed to experimental adult hard foraging conditions have lower AUC (mmol l^{-1}, mean±s.e.m.) values following an intra-peritoneal glucose administration, indicating higher glucose tolerance (LMM, $F_{1,111}$=5.83, P=0.02). Glucose tolerance was measured as incremental AUC after an exogenous glucose challenge; adjacent areas resulting from four glucose measurements were added after subtracting baseline glucose levels. Higher AUC values represent lower glucose tolerance, whereas lower AUC values correspond to higher glucose tolerance. Data shown correspond to least square means from a model including ambient variables, body mass, sample age, and the experimental treatments; AUC values were transformed to a standard normal distribution prior to analysis and least square means shown here were back transformed. Glucose tolerance test was performed in 122 adult zebra finches.

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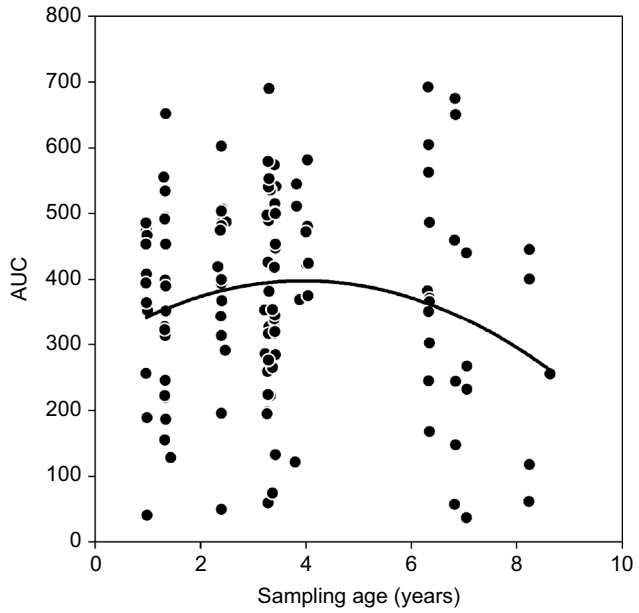

Fig. 3. AUC (mmol l^{-1}) after a glucose challenge was lower in birds sampled at younger and older ages, indicating young and old birds had higher glucose tolerance (LMM, $F_{1,111}$=4.60, P=0.03). Glucose tolerance was measured as incremental AUC after an exogenous glucose challenge; adjacent areas resulting from four glucose measurements were added after subtracting baseline glucose levels. Higher AUC values represent lower glucose tolerance whereas AUC values correspond to higher glucose tolerance. Glucose tolerance test was performed in 122 adult zebra finches.

from the model in Table 2). Correcting these models for left truncation following Therneau and Grambsch (2000) gave results slightly stronger as indicated by the estimate, albeit weaker in terms of statistical significance [quadratic term for >4 years: exp(coef)=0.819±0.122, z=−1.63, P=0.10]. Adding body mass to the model in Table 2 did not change the results, and we found no association between body mass and survival probability in this study (z=0.18, P=0.86).

In the same study population, high baseline glucose levels were previously found to be associated with reduced survival, raising the question of whether the association between survival and the AUC was independent of the association between survival and baseline glucose. The AUC was not correlated with baseline glucose (see above), and accordingly, including baseline glucose in the survival model in Table 2 showed that baseline glucose and AUC levels independently predicted survival [baseline glucose: exp(coef)=1.074±0.044, z=1.61, P=0.01]; other results reported (effect of the interaction between AUC squared and sampling age on survival) remained qualitatively unchanged.

Next, we analyzed whether the reduced survival of the birds exposed to experimental manipulations previously reported in this population (Briga et al., 2017) could be mediated by glucose tolerance as reflected in AUC values. To this end, we repeated the survival analysis in Table 2, but now including brood size and foraging effort treatments as fixed factors. This analysis revealed that the inclusion of these treatments had little effect on the association between AUC and survival (sampling age×AUC squared: exp(coef)=0.916±0.042, z=−2.08, P=0.038), indicating that the influence of the experimental manipulations on survival was not mediated through effects on glucose tolerance.

Survival probability was predicted by the interaction between robustness squared and sampling age, as we found for the AUC (Table 2; see Table S1 for left-truncated models). Similar to what we found for AUC, the quadratic association of robustness with survival was only present in old birds [>4 years: exp(coef)=0.99±0.004, z=−2.23, P=0.026; <4 years; exp(coef)=1.003±0.003, z=1.16, P=0.25; Fig. 4B]. There was no association of resilience with survival (Table 2; see Table S1 for left-truncated models).

Table 2. Cox proportional hazards (CPH) analyses exploring the links of glucose tolerance with survival, aviary was included as a random effect

Fixed effects	Coef.	exp(coef)±s.e.	z	P
Sampling age	**−0.491**	**0.612±0.083**	**−5.91**	**<0.0001**
AUC	0.100	1.105±0.245	0.41	0.680
AUC squared	**0.416**	**0.516±0.188**	**2.21**	**0.027**
AUC×Sampling age	−0.027	0.974±0.056	−0.48	0.630
AUC squared×Sampling age	**−0.095**	**0.909±0.041**	**−2.31**	**0.021**
Sampling age	**−0.954**	**0.385±0.197**	**−4.85**	**<0.001**
Robustness	**−0.323**	**0.724±0.134**	**−2.40**	**0.016**
Robustness squared	**0.012**	**1.011±0.005**	**2.25**	**0.025**
Robustness×Sampling age	**0.063**	**1.065±0.028**	**2.20**	**0.028**
Robustness squared×Sampling age	**−0.002**	**0.998±0.001**	**−2.11**	**0.035**
Sampling age	**−0.676**	**0.509±0.112**	**−6.02**	**<0.001**
Resilience	−0.134	0.875±0.085	−1.57	0.12
Resilience squared	0.006	1.006±0.004	1.42	0.16
Resilience×Sampling age	0.024	1.024±0.812	1.30	0.19
Resilience squared×Sampling age	−0.001	0.999±0.001	−1.25	0.21

In bold are fixed variables that explain variation on survival.

DISCUSSION

The capacity to maintain homeostasis is of general interest in evolutionary ecology as a potential indicator of fitness prospects, and may thereby contribute to our understanding of how fitness depends on age, behavior and environmental conditions. Therefore, the aim of this study was to evaluate whether glucose tolerance, as a proxy of the capacity to maintain homeostasis, was linked to survival.

We examined the effects of ambient and individual factors on the IP-GTT performance before testing for its effects on survival. Consistently with what has been reported in humans, at higher ambient temperatures the AUC levels were higher, and robustness and resilience were lower (Akanji and Oputa, 1991; Moses et al., 1997; Dumke et al., 2015; Antoine-Jonville et al., 2019), when compared with lower temperatures. The positive association between ambient temperature and the AUC may be the consequence of the lower metabolic rate exhibited at temperatures closer to thermoneutrality (e.g. Naya et al., 2018; Briga and Verhulst, 2021), when low energy turnover results in a decrease in glucose utilization in energy metabolism. Low metabolic rate at high temperatures could result from reduced energy demand from thermoregulation and/or a reduction in physical activity (Cooper et al., 2019). This scenario would be consistent with the observed negative association between baseline glucose and temperature, when baseline glucose is regulated at a low level in response to a reduced energy turnover (Montoya et al., 2018). Accordingly, in healthy humans, the positive link between AUC and ambient temperature is accompanied by low oxygen consumption and carbohydrate oxidation (Dumke et al., 2015).

Developmental conditions (rearing brood size) did not affect glucose tolerance, but birds exposed to increased foraging costs showed higher glucose tolerance (i.e. a lower AUC) than birds from the benign foraging condition. This result is in line with findings in

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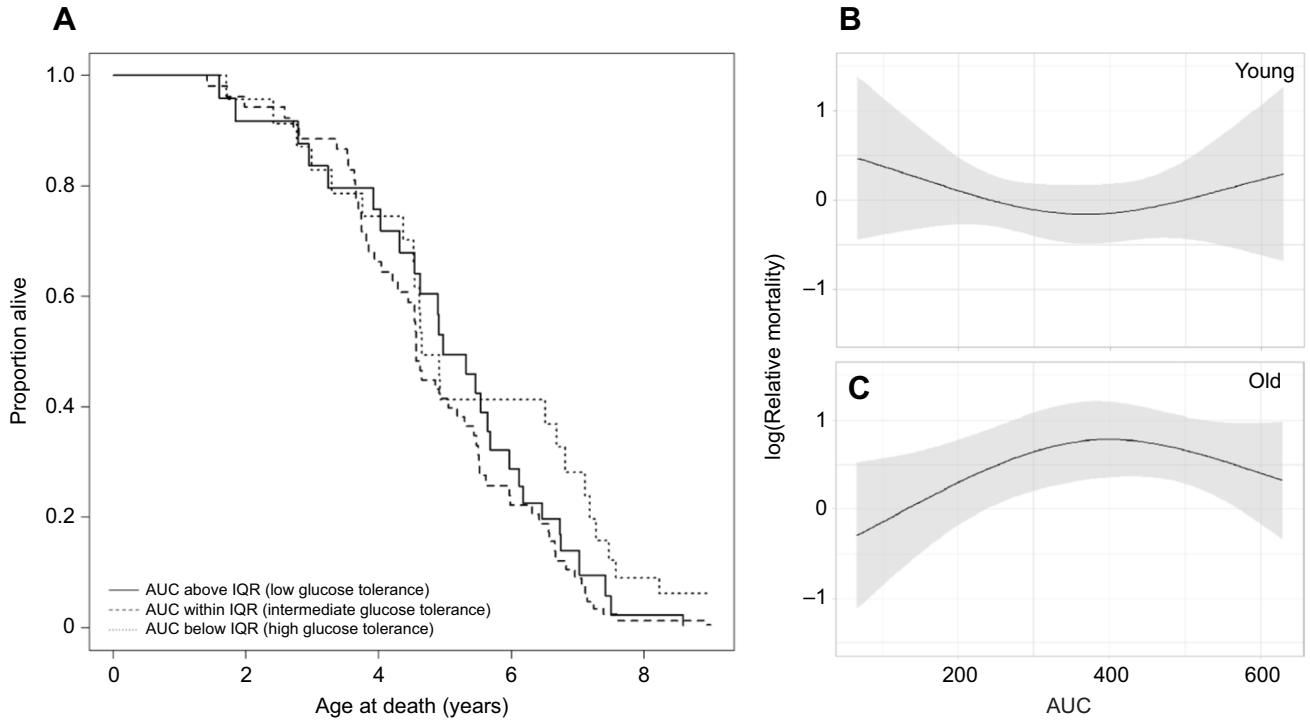

Fig. 4. Glucose tolerance predicts survival in old birds. (A) Birds with high glucose tolerance (i.e. low AUC mmol l^{-1}) had higher survival at old age than those with low glucose tolerance (i.e. high AUC mmol l^{-1}) (CPH, z=−2.31, P=0.021). Survival analyses were performed including AUC as a continuous variable but for illustrative purposes, we show data clustered in three groups: (1) high glucose tolerance: AUC below the interquartile range (IQR), dotted line; (2) low glucose tolerance: AUC above the IQR, solid line; and (3) intermediate glucose tolerance: AUC within the IQR, dashed line. (B) Glucose tolerance was not associated with mortality in young birds (<median age 3.3 years). (C) High glucose tolerance (low AUC) was linked to lower predicted mortality when compared with intermediate and low glucose tolerance, in old birds (>3.3 years). Note that survival analyses were performed with age as a continuous variable, but plotted separately as two groups for illustrative purpose only. Glucose tolerance was measured as incremental AUC after an exogenous glucose challenge; adjacent areas resulting from four glucose measurements were added after subtracting baseline glucose levels. Glucose tolerance test was performed in 122 adult zebra finches.

humans, where a reduced AUC is even found 24 h post-exercise (Bonen et al., 1998). Birds living with increased foraging costs (higher physical activity) throughout adult life may have a higher energy diurnal turnover than birds in benign foraging conditions (Hambly et al., 2004; Yap et al., 2017; Jimeno et al., 2018c), as indicated by our earlier finding that baseline glucose was higher in birds facing higher foraging costs (Montoya et al., 2018). Therefore, it seems likely that those birds used the extra glucose for energy storage as high foraging costs increase the utility of an energy dose, because larger effort is required to obtain it, when compared with the low foraging costs condition (Koetsier and Verhulst, 2011). Unfortunately, verifying the use of extra glucose for energy store in terms of mass would be difficult given the small glucose doses used in our study. Interestingly, robustness and resilience of the IP-GTT performance were not associated with adverse developmental or adult conditions, which suggests that the AUC is a more sensitive estimate of the individual's capacity to deal with the glucose challenge than resilience or robustness (Ukraintseva et al., 2016).

Individuals sampled at old and young ages appear to present better capacity to regulate glucose levels, independently of variation in mass and brood size treatment when compared with individuals sampled at middle age. This result contrasts with our prediction that the capacity to regulate glucose will deteriorate with age. However, the current finding is based on cross-sectional data, which do not reflect longitudinal changes within individuals in a trait when mortality is not random with respect to that trait (e.g. van de Pol & Verhulst, 2006) as found here (see below). The quadratic age pattern in glucose tolerance may be the outcome of a combination of processes. The initial AUC increase with age may result from a senescence effect, similar to that reported for other physiological markers in humans exposed to challenging conditions (Ukraintseva et al., 2021). The subsequent decrease in AUC with age may result from selective disappearance of individuals with a high AUC from the population (see below). These two processes together would result in the observed pattern of AUC decrease among older individuals, but longitudinal data are required to test this hypothesis.

Old individuals with high glucose tolerance (i.e. low AUC) showed higher survival than old individuals with intermediate or low glucose tolerance. Robustness of the regulatory response was similarly associated with survival probability, while the association with resilience was not significant. This combination of results suggests that the ability to minimize the increase of glucose levels following glucose administration better predicts survival probability than the ability to regulate glucose level back to baseline during the IP-GTT, as has been suggested also for humans (Arbeev et al., 2019). Interestingly, the association between glucose tolerance and survival was only evident at old ages. Young birds might be less vulnerable to lower glucose tolerance owing to a higher redundancy in other physiological processes contributing to maintain overall homeostasis (Boonekamp et al., 2015), as also evidenced by increase in mortality with age (Briga et al., 2017). Hence, variation in glucose tolerance measured at younger ages may thereby be not informative on mortality causes at old ages. Alternatively, causes of death and their dependence on homeostatic capacity may be age dependent.

Considering that the adult foraging treatment was found to affect glucose regulation (this study), as well as survival (Briga et al., 2017), we explored whether adding the experimental treatments to the survival model would attenuate the association between glucose tolerance and survival. However, this was not the case, suggesting that the effect of adult foraging treatment on survival probability in this population (Briga et al., 2017) was mediated by mechanisms other than the capacity to maintain homeostasis as characterized by glucose tolerance.

High baseline glucose was previously associated with low survival in this population (Montoya et al., 2018). High baseline glucose can arise owing to various causes, such as metabolic demands, stress response, and reduced ability to regulate glucose among others. Here, we implicitly tested the contribution of the latter possibility, and found that there was no association between baseline glucose and the AUC (as in humans, e.g. Le Floch et al., 1990). Furthermore, the effect of glucose tolerance on survival of old birds was independent of baseline glucose level. This leads us to dismiss low regulation capacity as an explanation for the association between baseline glucose and survival. Hence, individual variation in glucose tolerance (which is 50% repeatable; Montoya et al., 2020) may constitute a new approach to estimate individuals' vulnerability to challenges such as chronic social stress (Carneiro-Nascimento et al., 2020), infectious diseases (McGraw et al., 2020) and other conditions that may increase energy turnover under natural conditions. Such an approach would be consistent with the idea that resilience is strongly affected by the individual's metabolic response to a challenge (van der Kooij, 2020). Compared with baseline glucose (which is 30–54% repeatable), glucose tolerance appears to be more sensitive to fluctuation in ambient temperature and age-dependent hazards at older ages, but less sensitive to early-life adverse conditions (Montoya et al., 2018, 2020). Using estimates of individuals' frailty under ecologically relevant conditions might be particularly pertinent to better understand factors linked to variation in the capacity to cope with changing environments. Therefore, this new approach warrants further research with wild animals.

We hypothesized that glucose tolerance could provide information on the capacity to maintain homeostasis, which is typically compromised during senescence. On the one hand, our findings confirmed this hypothesis, because glucose tolerance was a predictor of mortality in individuals sampled at old ages. On the other hand, at least part of the variation in glucose tolerance can be interpreted as the consequence of variation in energy turnover. This result gives rise to an alternative interpretation of the association between survival and glucose tolerance, with variation in metabolic rate being the responsible for such an association. In this case, individuals with high metabolic rates, which would result in a strong glucose tolerance (lower AUC), would show improved survival. This interpretation remains to be tested, but relevant information available from this same species argues against it. Firstly, the dependence of survival on acute increases in corticosterone (Jimeno et al., 2018a), which relates strongly to simultaneous metabolic rate (Jimeno et al., 2018b), shows the opposite effect for males (low survival when corticosterone is high) and no association in females. Secondly, nocturnal metabolic rate, at temperatures either in or below the thermoneutral zone, did not predict survival (Briga and Verhulst, 2021). Thus, we tentatively conclude that the observed association between glucose tolerance and survival is the consequence of individuals with high capacity to sense nutrients (i.e. exogenous glucose) and maintain homeostasis showing better survival. At the same time, we recognize that complementary measures of homeostatic capacity are needed to draw more definite conclusions on the role of homeostatic capacity in causing variation in survival.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: B.M., S.V.; Methodology: B.M., S.V.; Formal analysis: B.M., M.B., S.V.; Investigation: B.M., M.B., B.J.; Writing - original draft: B.M.; Writing - review & editing: M.B., B.J., S.V.; Supervision: S.V.; Funding acquisition: S.V.

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Data availability
All data used in this manuscript can be accessed from the Dryad digital repository (Montoya et al., 2022): https://doi.org/10.5061/dryad.8pk0p2nq2

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