Exposure to food insecurity increases energy storage and reduces somatic maintenance in European starlings

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Abstract

Birds exposed to food insecurity—defined as temporally variable access to food—respond adaptively by storing more energy. In order to do this, they may reduce energy allocation to other functions such as somatic maintenance and repair. To investigate this trade-off, we exposed juvenile European starlings (Sturnus vulgaris, n = 69) to 19 weeks of either uninterrupted food availability, or a regime where food was unpredictably unavailable for five hours on five days each week. Our measures of energy storage were repeated measurements of mass, and fat score at the end of the treatment. Our measures of somatic maintenance were growth rate of a repeatedly plucked tail feather, and erythrocyte telomere length, which we measured five times by analysis of the terminal restriction fragment. The insecure birds were heavier at all measurement points, but by an amount that varied across time points. They also had higher fat scores. We found no evidence that they consumed any more food overall, though our food consumption data was incomplete. Plucked tail feathers regrew more slowly in the insecure birds. Telomere length was reduced in the insecure birds, specifically, in the longer percentiles of the within-individual telomere length distribution. We conclude that increased energy storage in response to food insecurity is achieved at the expense of investment in somatic maintenance and repair.

Key words: food insecurity, insurance hypothesis, somatic maintenance, telomeres, birds, starlings
Introduction

When birds such as starlings are exposed to food insecurity—defined as temporally variable access to food—they respond by storing fat and gaining body mass [1–6]. This is an adaptive response: the greater the risk of a period of shortfall, the larger the energy buffer it is optimal to store [7–10]. Something very similar may occur in humans, at least in females: experience of food insecurity, measured by questionnaire, is associated with higher body mass index [8,11]. It has been widely assumed that the mechanism underlying food-insecurity driven mass gain is increased food consumption during the times when food is available [12–14]. However, the empirical evidence does not currently support this assumption. In food insecurity experiments, birds can gain weight whilst not increasing their food consumption, or even whilst decreasing it [3,5,6,15]. Likewise, food-insecure women have higher body mass indices without apparently consuming any more calories [16–20]. Another possibility is that food-insecure individuals sequester more energy for fat storage by reducing their energy expenditure rather than increasing their intake. In related and relevant work, Wiersma and Verhulst [21] showed that when foraging was made more costly by mixing food with chaff, zebra finches decreased their daily energy expenditure, despite the greater time spent foraging.

There are several ways that an animal might reduce energy expenditure. Zebra finches have been shown to reduce energy expenditure in response to food insecurity [22], though recent evidence from European starlings was inconclusive [5]. Beyond physical activity, animals may down-regulate investments in somatic maintenance and repair. In zebra finches, Marasco et al. [23] found that long-term exposure to food insecurity increased the rate of accumulation of DNA damage (as measured by 8-hydroxy-2′-deoxyguanosine). Wiersma and Verhulst [21] found that zebra finches whose foraging costs were increased regrew a plucked tail feather more slowly than control birds. Another possible marker of somatic maintenance is telomere length (TL). Telomeres are repetitive DNA sequences at the ends of chromosomes that serve to maintain chromosome integrity [24]. They gradually shorten with age due to end-replication problems and other processes [25], shortening that is accelerated by oxidative stress [review; 26]. Across non-human vertebrates, shorter TL or accelerated telomere shortening is associated with ecological challenges such as infection, high competition, poor food or harsh abiotic conditions [meta-analysis; 27]. In nestling starlings, nutritional shortfall and increased begging effort accelerate telomere shortening [28]. Individuals can invest in maintaining TL through antioxidant defences [29]. Thus, change in TL in a proliferating tissue such as erythrocytes can be used as an index of investment in somatic maintenance and repair.

In the present study, we exposed groups of captive wild-caught juvenile European starlings to an extended period (19 weeks) of either food insecurity, or constant food availability. Our method of imposing food insecurity was similar to that of several earlier studies [2,23,30]: the removal of access to any food for a five hour period in the fifteen hour day, whose timing during the day varied randomly. In the present case, this was done five days out of seven, with uninterrupted access to food on the remaining two days. Note that this manipulation introduces both restriction of food access, and temporal unpredictability, to the insecure birds compared to the controls. It was not the aim of this study to distinguish the effect of unpredictability from that of restriction, as some other studies have done [1,3]. Food insecurity in the wild may typically involve both, and we were simply seeking a food insecurity regime sufficient to affect the birds in naturalistic manner. We measured body mass repeatedly, as well as fat scores at the end of the treatment period. In addition, we measure two potential markers of somatic maintenance and repair, induced feather regrowth and erythrocyte telomere length (TL). We measured TL by terminal telomere restriction fragment
analysis. This has the advantages, compared to the popular qPCR relative telomere length assay [31], of higher precision, and providing, for each sample, a distribution of the lengths of the telomeres present, not just a single estimate of central tendency [32]. We also gathered some information on food consumption, though for logistical reasons the consumption data did not cover every day of the study period. Our general hypothesis was that food insecurity would produce an increase in energy storage and decreased expenditure on somatic maintenance and repair. Hence, we predicted body fat and mass would increase, whilst the rate of feather regrowth and TL would decrease, under food insecurity compared to the control treatment.

Methods
Ethics and permissions
This study was completed under UK Home Office licence 70/8089 (licence holder Melissa Bateson) and with approval of the Animal Welfare Ethical Review Board at Newcastle University. Capture of birds from the wild was done with landowner permission under Natural England permit number 2016-S7171-SCI-SCI. A copy of the ARRIVE guidelines 2.0 essential items check list [33] is included as Supporting Information.

Birds and aviaries
We captured 70 European starlings in Northumberland over four days in October 2016 using a whoosh net at a site we had been baiting. The number of birds was limited by aviary capacity constraints, but was several-fold larger than the numbers of animals used in comparable previous experiments (typically 6 – 24; [3–6,21,34]). Juvenile status (having hatched in Spring 2016) was still recognisable from plumage, and only juveniles were retained. Birds were transported in cloth bags to the laboratory (approximately 30 minutes), where they were weighed and inspected. Sex was established from visual appearance (and subsequently confirmed genetically, although only after treatment allocation). An initial blood sample was taken (see blood sampling, below); one tail feather pulled (see feather regrowth, below); and a numbered plastic leg ring fitted. Birds were also treated with topical Ivermectin to kill common parasites. Birds were then released into one of four indoor aviaries, where they remained for the duration of the experiment. The aviaries varied slightly in size, with width 239-246 cm, depth 209-219 cm, and height 240cm. The light cycle of the aviaries was 15L:8D with dim lighting simulating dawn/dusk during the first/last 30 mins of the light period. Drinking water was available at all times and environmental enrichment was provided in the form of rope perches, water baths and wood shaving substrate. Diet throughout the study was a mixture of commercially available dry cat food (Royal Canin Ltd.), turkey crumb (Special Diets Services ‘Poultry Starter (HPS)’), and insect mix for birds (Orlux insect patée). Birds were left to settle in their aviaries with ad libitum food for 11-19 days prior to the beginning of the experimental treatment.

Catching for weighing or blood sampling, as outlined below, was done in the dark one hour prior to the birds’ dawn, and birds were placed into cloth bags until they were processed and re-released into their aviaries.

One bird was euthanised prior to the beginning of the treatment, owing to lethargy and very low body weight. This left a final sample of 69 birds, assessed as 29 females and 40 males. On conclusion of the experiment, birds were given a period of ad libitum food, inspected by a veterinarian, transported to the site of capture in cloth bags, and released.

Experimental treatments
Two aviaries each were assigned to the two experimental treatments (‘insecure’ and ‘control’). Assignment was by alternation within sex, on removal from the bags, and so was effectively random apart from sex balancing. This produced 35 birds (20 male) in the insecure treatment and 34 (20 male) in the control treatment. For five days a week (Monday to Friday), food was provided in automated pet feeders (Andrew James Ltd; three per aviary). These worked by sequentially revealing four compartments at pre-programmed times. For the control treatment, all compartments were full of food. Thus, although the feeders moved from compartment to compartment at the same times as for the insecure treatment, food was always available. For the insecure treatment, one compartment was empty, and thus no food was available for five hours out of the day. The timing of the period without food was varied pseudo-randomly from day to day, but was the same for the two aviaries in the insecure treatment. Food deprivation could begin at any hour within the period of full light, and could last until dusk (i.e. the earliest onset of the 5 h period was after the 30 minutes of dawn, and the latest end of the 5 h period was at the beginning of the 30 minutes of dusk). Food in each non-empty compartment was sufficient that it never ran out. On the remaining two days of the week, uninterrupted food access was provided to both aviaries all day in open bowls. During week 9, uninterrupted food access was provided to both groups every day, as the facility was closed for a public holiday. The experimental treatment was continued for a total of 19 weeks.

Body mass and fat scoring

Birds were weighed before dawn. Body masses and fat scores by the main scorer were not made blind to treatment. At all weighing points, body mass was measured by placing the bird in a plastic cone on a digital scale measuring to a resolution of 0.1 g. Body mass was measured on arrival, immediately prior to the beginning of the experimental treatment (henceforth baseline), then after 2, 5, 8, 11, 14, 17 and 19 weeks of treatment. In addition, all birds were manually fat scored at week 19 (0-8, Biometrics Working Group system [35]) by CA, who was not blind to treatment. Fat score was positively correlated with mass (r = 0.58, p < 0.001). A subset of 14 birds was also fat scored independently by a different, experienced avian fat scorer blind to treatment. The intra-class correlation coefficient (ICC1) for the two raters was 0.75 (95% CI 0.72 – 079).

Food consumption

Food consumption was estimated for four days out of every seven by weighing the food remaining in the automated feeders. Due to logistical constraints, it was not possible to weigh the food on Fridays, Saturdays or Sundays. Thus, the food consumption data are incomplete and do not cover the two days per week when the insecure birds had ad libitum food. Food consumption was only measured at the aviary level. Food weighings were not blind to treatment. We averaged across the four days of each week to produce one consumption number for each aviary in each week, and converted this to g per bird per day to correct for the different numbers of birds in each aviary.

Feather regrowth

On capture, we removed the left outer retrix (tail feather) by grasping the rachis with blunt-ended forceps and gently pulling until the feather released. This was repeated after 5 and 17 weeks of treatment, by which times the pulled feather had largely grown back. The length of the regrowing feather was measured in mm using digital callipers, from the base of the pin to the most distal point of the feather tip, after 2, 5, 8, 11, 14, 17 and 19 weeks of treatment. These measurements were blind to treatment. At week 17, three birds had feather lengths substantially shorter than they had
been at week 14. These were assumed to represent breakage or accidental loss and excluded from analysis.

**Telomere length (TL)**

TL was measured in erythrocytes by telomere restriction fragment analysis under non-denaturing conditions. Blood samples (around 140 μl) were taken by puncture of an alar vein with a 25-gauge needle and collection into capillary tubes. Samples were transferred to EDTA-treated plastic tubes on ice. They were then centrifuged to separate cells from plasma (10 minutes at RCF 1400 g), and pellets of cells frozen to -80°C. Blood samples were taken on arrival (henceforth baseline; note that this is two weeks earlier than the baseline date for mass), and after 2, 8, 14 and 19 weeks of treatment.

TL analysis followed the methods of Bauch et al. [36]. In brief, we washed the cells and isolated DNA from 5 μl of erythrocytes using CHEF Genomic DNA Plug kit (Bio-Rad, Hercules, CA, USA). Cells in the agarose plugs were digested overnight with Proteinase K at 50°C. Half of a plug per sample was restricted simultaneously with HindIII (60 U), HinfI (30 U) and MspI (60 U) for ~18 h in NEB2 buffer (New England Biolabs Inc., Beverly, MA, USA). The restricted DNA was then separated by pulsed-field gel electrophoresis in a 0.8% agarose gel (Pulsed Field Certified Agarose, Bio-Rad) at 14°C for 24h, 3.5V/cm, initial switch time 0.5 s, final switch time 7.0 s. For size calibration, we added 32P-labelled size ladders (DNA Molecular Weight Marker XV, Roche Diagnostics, Basel, Switzerland; NEB MidRange PFG Marker I, New England Biolabs, range 15–242.5 kb). Gels were dried (gel dryer, Bio-Rad, model 538) at room temperature and hybridized overnight at 37°C with a 32P-endlabelled oligonucleotide (5'-CCCTAA-3')4 that binds to the single-strand overhang of telomeres of non-denatured DNA. Subsequently, unbound oligonucleotides were removed by washing the gel for 30 min at 37°C with 0.25x saline-sodium citrate buffer. The radioactive signal of the sample specific TL distribution was detected by a phosphor screen (MS, Perkin-Elmer Inc., Waltham, MA, USA), exposed overnight, and visualized using a phosphor imager (Cyclone Storage Phosphor System, Perkin-Elmer Inc.).

TL distributions were quantified using IMAGEJ (v. 1.38x). The TL parameters potentially relevant to aging and somatic state are not just average TL, but aspects of an individual’s TL distribution (for example, the length of the shortest or longest telomeres). We therefore calculated the mean of the TL distribution (i.e. henceforth aTL), and additionally the percentiles, in 5% intervals, from 10% to 90%. For each sample the limit at the side of the short telomeres of the distribution was lane-specifically set at the point of the lowest signal (i.e. background intensity). The limit on the side of the long telomeres of the distribution was set lane-specifically where the signal dropped below Y, where Y is the sum of the background intensity plus 10% of the difference between peak intensity and background intensity. The coefficient of variation of a control sample run on 15 gels was 6%. The intra-class correlation coefficient (ICC) across individuals was 0.77, including treatment week as a fixed factor. This represents a minimum estimate of the technical repeatability of the TL measurements. All telomere measurements were blind to treatment.

**Statistical analysis**

Data were analysed in R, version 3.6.0 [37], using linear mixed models with R packages ‘lme4’ and ‘lmerTest’. Model estimation used restricted maximum likelihood. Significance testing used Satterthwaite’s method with α = 0.05. Models of experimental effects used insecurity status as the fixed predictor, where this status was control for all birds at baseline, and insecure for the insecurity groups subsequent to the onset of the experimental treatment. Taking the data from the onset of
the experimental treatments onwards and using treatment group as the fixed predictor produces
very similar results. Preliminary inspection revealed week-to-week changes with no linear trend,
especially for mass (perhaps due to temperature and seasonal variation). We therefore included
treatment week as a fixed factor rather than a continuous covariate. The interaction between
treatment week and insecurity was also included. The distributions of residuals were checked and
found satisfactory for the assumptions of the models. For binary comparisons of means, we report
Cohen’s d as measures of effect size.

Models for mass, TL and feather regrowth included random effects of bird to account for repeated
measures. Adding aviary as an additional level of random effect did not improve AIC or change
results, and hence was not included in the analyses presented below. For TL, in a first model, the
outcome variable was aTL. In a follow up model, we used all available percentiles of the TL
distribution. For this model, the fixed predictors were insecurity, week, percentile, and all possible
interactions, with random effects of sample identity and bird.

In addition to analyses of individual outcomes, we present meta-analyses, in which we combine the
evidence for a treatment effect from the two measures of energy storage (mass and fat score), and
the two principal measures of somatic investment (average telomere length and feather regrowth).
For these models, we standardized the dependent variables for comparability of parameter
estimates, and excluded the interaction between week and insecurity. Meta-analyses were
conducted using R package ‘metafor’.

Results

Mass and fat scores

Mass at baseline did not differ significantly between the treatment groups (control: mean 75.20g, se
0.72; insecure: 74.80g, se 0.76; t = -0.16, p = 0.87). Insecure birds were heavier than control birds at
all time points after the onset of the treatment, by varying amounts (figure 1A). The main effect of
insecurity was marginally non-significant (F(1, 505.38) = 3.24, p = 0.07), but there was a significant
interaction between insecurity and week (F(6, 469.37) = 2.22, p = 0.04). The mass difference by
insecurity status was substantial at weeks 14 (1.76 g; d = 0.40, 95% CI -0.08-0.89) and 19 (2.08 g; d =
0.48, 95% CI -0.01-0.97) and negligible at, weeks 5 (0.13 g; d = 0.03, 95% CI -0.45-0.51) and 8 (0.10 g;
d = 0.02, 95% CI -0.46 – 0.50).

Fat scores at week 19 were significantly higher for the insecure group (mean 3.59, se 0.13) than the
control group (mean 3.06, se 0.14; t = 2.76, p = 0.01; d = 0.66, 95% CI 0.17 – 1.15; figure 1B).
Figure 1. Effects of experimental treatment on mass, fat and food consumption. A. Mass change from baseline ± 1 se, by treatment across the experimental period. B. Fat scores after 19 weeks of treatment, by treatment. Points represent birds. C. Food consumption (g per bird per day), by treatment. Points represent aviary weeks.

Food consumption

We calculated food consumed per bird at the aviary level, as described in Methods (i.e. there was one data point per aviary per week). We fitted a model with food consumed per bird as the outcome, and insecurity, week and their interactions as fixed predictors. The main effect of insecurity was not significant (F(1, 2) = 0.22, p = 0.68), and the interaction between week and insecurity was marginally non-significant (F(17, 34) = 1.91, p = 0.05). The insecure birds consumed slightly less per bird overall (control: 10.48 g, se 0.17; insecure: 10.01 g, se 0.33; d = -0.25, 95% -0.73 – 0.22; figure 1C).

Telomere length

Mean aTL at baseline was 17351 bp (sd 1032, range 15110 – 20179). Individual TL showed high degrees of consistency over time; for example, the correlation matrix of aTL across individuals at the various time points is shown in table 1. Correlations over time for percentiles of the TL distribution were similar. On average, individuals’ TL shortened by 142 bp (sd 522) between baseline and the final TL measurement point 21 weeks later (t = -2.26, p = 0.03).

Table 1. Correlations across individuals for average TL at different time points.

|           | Week 2 | Week 8 | Week 14 | Week 19 |
|-----------|--------|--------|---------|---------|
| Baseline  | 0.76   | 0.75   | 0.77    | 0.87    |
| Week 2    | 0.76   | 0.77   | 0.75    |         |
| Week 8    | 0.80   | 0.80   |         |         |
| Week 14   |        |        | 0.85    |         |

At baseline, aTL did not differ significantly between treatment groups (control: mean 17420, se 179; insecure: mean 17285, se 187; t = 0.52, p = 0.61). In the model using aTL as the outcome variable, the main effect of insecurity was marginally non-significant (F(1, 326.78) = 3.03, p = 0.08). The interaction between insecurity and week was not significant (F(3, 262.43) = 1.30, p = 0.27). Figure 2A shows aTL by treatment group at each measurement point; insecure birds had shorter aTL than
control birds at all time points. The difference between the two groups was largest at week 2 (-516 bp; d = -0.45, 95% CI -0.96 – 0.06) and smallest at week 14 (-204 bp; d = -0.17, 95% CI -0.65 – 0.30).

We followed up this analysis with a model using the full range of percentiles of the TL distribution. The main effect of insecurity was not significant in this model (F(1, 298.3) = 2.57, p = 0.11), and neither was the main effect of week (F(4, 244.2) = 1.53, p = 0.19). There was however a significant interaction between insecurity and percentile (F(16, 5257.6) = 11.04, p < 0.001). No other interactions were significant. As figure 2B shows, the insecure birds had shorter TL at the longer percentiles of the TL distribution.

Figure 2. Effects of insecurity on telomere length. A. Average TL by insecurity status though the treatment. Error bars represent one standard error. The dotted vertical line represents the onset of the treatments. B. Difference between insecure and control birds by percentile of the TL distribution, collapsed across the weeks after the onset of the treatment. Data represent difference in marginal means (± 1 se), estimated from the statistical model. A negative number indicates shorter TL in the insecure birds.

**Feather regrowth**

For feather regrowth, as well as an expected large effect of week (F(6, 363.84) = 147.96, p < 0.001), there was a significant effect of treatment (F(1, 66.37) = 5.40, p = 0.02). Birds from the insecure groups had slightly but consistently shorter feathers at all time points other than the final one (figure 3). The interaction between treatment and week was not significant (F(6, 363.84) = 0.35, p = 0.91).

Averaging across the measurement points, insecure birds had an average feather length of 53.90 mm (se 1.01) compared to 56.20 mm (se 0.42) for the control birds, corresponding to an effect size (Cohen’s d) of -0.50 (95% CI 0.01 – 0.99).
Figure 3. Length of regrowing tail feathers (mm) by insecurity and time point. The pulling of the feather is indicated by the vertical solid lines. The beginning of the treatment phase is shown with a vertical dotted line. Shown are estimated marginal means plus or minus one standard error. At weeks 17 and 19, the data are overlapping.

Meta-analysis

In a fixed effects meta-analysis of the two measures of energy storage, mass and fat score, there was a significant positive effect of insecurity (figure 5; $B = 0.27$, se 0.09, 95% $z = 2.79$, $p < 0.001$). For the measures of somatic investment, average TL and feather regrowth, there was a significant negative effect of insecurity (figure 5; $B = -0.16$, se 0.06, 95% $z = -2.86$, $p < 0.001$).
Figure 5. Meta-analysis of study measures. Squares represent standardized effect sizes, and whiskers represent 95% confidence intervals. Diamonds represent pooled effect sizes and their 95% confidence interval from a fixed-effects meta-analysis model.

Discussion

We experimentally exposed groups of young starlings to food insecurity or uninterrupted food access over a period of more than four months. Overall, our results support the hypothesis that the birds experiencing food insecurity increased energy storage, and reduced somatic investment and repair. When the evidence from fat scores and masses was combined meta-analytically there was a clear pattern of increased energy storage, though the effect on mass considered separately was significant only in interaction with time point. On the somatic maintenance side, again the pattern of reduced investment was clearer when TL and feather regrowth were combined meta-analytically. Considered separately, the effect of insecurity on TL was significant only in interaction with percentile of the TL distribution: the longer telomeres were those affected.

Our findings that food insecurity increased energy storage conceptually replicate earlier findings in starlings and other passerine birds [3–6,38]. The insecurity effect on mass varied from week to week; when averaged over all the weeks, the effect size was small. This is consistent with our recent findings from a series of experiments using a different method of inducing food insecurity in starlings. There, we found evidence for mass gain under food insecurity overall, but with effects that varied in magnitude from experiment to experiment and were null in some experiments [5]. How successful the laboratory protocols are at simulating natural food insecurity is not clear; it may be that they underestimate the magnitude or reliability of the shifts in the wild, given, for example, that
in the current experiment, birds would have been able to learn that the absence of food is always short-lived.

There are several non-mutually exclusive mechanisms that could explain how food insecurity induces fat storage. The first is that food insecure birds consume more food in the periods when food is available [12–14]. We found no evidence for increased food consumption. This result is not definitive: in the present experiment, we only measured food consumption on four days out of every seven, and only at the coarse level of the whole aviary. Thus, we cannot exclude that the food insecure aviaries consumed more food than the control aviaries on the two ad libitum days per week where food consumption was not monitored. Nonetheless, the non-significant trend we observed was in the direction of insecure birds eating less rather than more. This finding is consistent with a number of other avian studies where food consumption was measured more completely, in which food insecure birds gained weight despite eating no more food, or less food [3,5,6,15]. It is also consistent with the human evidence that food insecure women gain weight without apparently consuming any more calories, [16–20], though those studies suffer from the limitation that food consumption is self-reported.

A second possible mechanism is that food insecure birds assimilate more of the potential caloric content of the food they do consume. In our previous study in the same species [5], we used bomb calorimetry to measure the energy density of guano. We found lower energy density of guano in food insecure birds, suggesting greater assimilation of the caloric content. We did not collect guano in the present experiment, and hence have no information on whether assimilation was increased, though this is plausible given previous findings [5,39].

Third, food insecure birds may reduce energy expenditure on other functions. Our findings on feather regrowth and TL suggest in particular that energy allocation to somatic maintenance and repair was reduced. These findings are consistent with Wiersma and Verhulst’s [21] demonstration of reduced feather regrowth in zebra finches for whom foraging had been made less profitable, and the evidence from Marasco et al. [23], also in zebra finches, of a faster accumulation of DNA damage over time in birds exposed to a food insecurity regime very similar to the present one. Whereas our choice of energy storage measures was straightforward, in that mass and fat score are the directly relevant quantities, our choice of feather regrowth and TL was opportunistic. There were other potential measures we could have chosen but did not, such as DNA damage or immune function.

Previous studies suggest food insecurity may have similar negative effects on those measures [15,23]. The choice of measures in the present case was dictated by convenience and our prior expertise in telomere dynamics [40–42]. The fact that both our chosen measures showed some evidence of a reduction under food insecurity was either fortunate, or suggests that reduction of investment under food insecurity is detectable across a range of possible markers of somatic investment. Such reduced investment would provide a general pathway to explain the reliable associations between food insecurity and subsequent poor health in humans [43,44]. It is, however, difficult to reconcile with findings that long-term exposure to a food insecurity regime increased life expectancy in zebra finches [45]. We note also that we did not measure other components of energy expenditure, such as movement, thermoregulation [46], preparation for reproduction, or song and song learning [47], that could have also been reduced under food insecurity.

Our investigation of TL under food insecurity was notable for its high precision, compared to many other avian TL studies. This precision arose from measuring TL five times on the same individuals, and using the terminal restriction fragment approach rather than the more widespread qPCR assay [see 32 for discussion of alternative TL measurement methods]. This method has several advantages. First, as used here it excludes interstitial telomere sequences, which can be numerous and variable
between individuals in birds. Thus, it provides a clean measure of terminal TL, which is the
parameter of interest. Second, using this method we were able to characterise the absolute lengths,
in base pairs, of telomeres in the European starling, whereas our previous work [40,41] reported
only relative abundance of the telomeric sequence. The average TL for the whole sample, 17351
base pairs, falls squarely within the range observed in birds, fairly similar to the values seen in blue
tits and zebra finches measured by the same method [48]. Third, measuring the terminal restriction
fragment provides a distribution of TL for each sample. This revealed that, though the effect of food
insecurity on average TL was non-significant, there was an interaction between insecurity and
percentile of the TL distribution, with insecurity appearing to shorten the longest telomeres within
individuals. In common terns, Bauch et al. [49] found that the length of the longest telomeres was a
better predictor than average telomere length of survival and reproductive success. Bauch et al.
suggest that this is due to the effects of environmental stressors being most visible in the longest
percentiles of the TL distribution, where telomeres shorten fastest in absolute terms. Our findings
represent a direct corroboration of this claim.

Our repeated measurement of TL also allowed us to characterise TL dynamics, albeit that the
timescale was short for examining age-related shortening given the rate at which TL changes after
early life. As in other studies using high-precision methods, TL was individually highly consistent over
time, with those individuals with long average TL at the beginning of the study generally having long
TL at the end [50]. Despite the restricted study period, we were able to observe TL shortening. For
average TL over the study period, the mean loss was 142 bp (se 66.9; by treatment it was 209 bp for
the insecure birds (se 66.3), and 77 bp for the control birds (se 115)). Averaging across the
treatments suggests an annual shortening rate of around 350 bp/year. This is in the range estimated
for other passerine birds [51], albeit that our birds were young and likely to be losing TL faster than
the whole-life rate.

Our results confirm that when faced with food insecurity, starlings can respond adaptively by
increasing energy allocated to fat storage, even without taking in any more food overall. At the same
time, they reduce allocation to somatic maintenance and repair. The costs of doing this are real and
measurable, in terms of slowed feather regrowth and erosion of the longest telomeres. Over time,
such reduced investments would presumably have measurable impacts on health. Classical
theoretical work on optimal energy reserves treated increased predation risk as the fitness cost of
fat storage [9,52,53]. The present work suggests that increased predation risk does not adequately
capture all of the costs, since energy intake is limited, and the energy to fund storage must be
diverted from other fitness-relevant functions.

Data availability
Code and raw data relating to this study are freely available in the Zenodo repository at
https://zenodo.org/record/5036419 (doi: 10.5281/zenodo.5036419).

Competing interests
The authors have no competing interests to declare.
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The ARRIVE guidelines 2.0: author checklist

**The ARRIVE Essential 10**

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

| Item | Recommendation | Section/line number, or reason for not reporting |
|------|----------------|-----------------------------------------------|
| **Study design** | 1 For each experiment, provide brief details of study design including:  
  a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated.  
  b. The experimental unit (e.g. a single animal, litter, or cage of animals). | Methods: Experimental treatments  
Methods: Experimental treatments |
| **Sample size** | 2  
  a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used.  
  b. Explain how the sample size was decided. Provide details of any a priori sample size calculation, if done. | Methods: Experimental treatments  
Methods: Experimental treatments |
| **Inclusion and exclusion criteria** | 3  
  a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established a priori. If no criteria were set, state this explicitly.  
  b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so.  
  c. For each analysis, report the exact value of n in each experimental group. | No exclusions  
Three feather measurements, see Methods: Feather regrowth  
Methods: Experimental treatments |
| **Randomisation** | 4  
  a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence.  
  b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly. | Methods: Experimental treatments  
Methods: Experimental treatments |
| **Blinding** | 5 Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis). | Methods |
| **Outcome measures** | 6  
  a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes).  
  b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size. | Introduction, Methods  
Methods: Statistical analysis |
| **Statistical methods** | 7  
  a. Provide details of the statistical methods used for each analysis, including software used.  
  b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met. | Methods: Statistical analysis  
Methods: Statistical analysis |
| **Experimental animals** | 8  
  a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight.  
  b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures. | Methods: Birds and avianes  
Methods: Birds and avianes |
| **Experimental procedures** | 9 For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including:  
  a. What was done, how it was done and what was used.  
  b. When and how often.  
  c. Where (including detail of any acclimatisation periods).  
  d. Why (provide rationale for procedures). | Methods  
Methods  
Methods |
| **Results** | 10 For each experiment conducted, including independent replications, report:  
  a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range).  
  b. If applicable, the effect size with a confidence interval. | Results (script and data also available)  
Results (script and data also available) |

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