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RESEARCH ARTICLE

Effects of experimental increase in insulin-like growth factor 1 on feather growth rate, moult intensity and feather quality in a passerine bird

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ABSTRACT

Moulting is a crucial, yet often overlooked life-history stage in many animals, when they renew their integumental structures. This life-history stage is an energetically demanding somatic growth event that has particular importance in birds because feathers play a crucial role in flight, insulation and communication. Somatic growth processes are regulated by the evolutionarily conserved peptide hormone insulin-like growth factor 1 (IGF-1). However, the role of IGF-1 in feather growth remains unknown. In this study, we captured 41 juvenile free-living bearded reedlings (*Panurus biarmicus*) that had started their first complete moult and brought them into captivity. Then, we manipulated their circulating IGF-1 levels using poly-(lactic-co-glycolid acid) microparticles (microspheres) that provide a sustained release of IGF-1. The treatment increased IGF-1 levels but did not affect the feather growth rate. However, 2 weeks after the treatment, birds in the increased IGF-1 group were moulting more feathers simultaneously than the controls and were at a more advanced stage of moult. Birds with experimentally increased IGF-1 levels had better quality feathers (measured by a lower number of fault bars) than the controls. These results suggest that an increase in IGF-1 does not speed up feather growth, but may alter moult intensity by initiating the renewal of several feathers simultaneously. This may shorten the overall moulting time but may imply costs in terms of IGF-1-induced oxidative stress.

KEY WORDS: IGF-1, Feather growth rate, Feather quality, Moult intensity, Life-history, Hormones, Bearded reedling, *Panurus biarmicus*

INTRODUCTION

Growth is a metabolically expensive process and is associated with severe costs (Werner and Anholt, 1993; Yearsley et al., 2004). These costs can result in important life-history trade-offs, with fast-growing individuals often suffering from higher mortality and a shorter lifespan than conspecifics growing at a more moderate pace (Metcalfe and Monaghan, 2003; Monaghan and Ozanne, 2018; Werner and Anholt, 1993). The significance of growth in shaping life-history trajectories has therefore been widely recognized (Gélin et al., 2016; Huot et al., 2014; Mangel and Stamps, 2001; Stamps, 2007). The term 'growth' often refers to structural and longitudinal growth events during embryonic and postnatal development. However, many organisms maintain growth throughout their lifetime (e.g. fish, amphibians and reptiles) and, even in animals that have reached their final adult body size, somatic growth events frequently occur. One particularly interesting example of such processes is moulting (also referred to as shedding in some taxa).

Moulting is an often overlooked somatic growth period that occurs regularly in adult organisms (Jenni and Winkler, 2020b; Payne, 1972). It serves in renewing the integumental structures (e.g. fur, scaled skin or feathers) covering the entire or most of the body surface, and similar to postnatal structural growth, it invokes serious fitness-related or physiological costs (Dawson, 2015; Jenni and Winkler, 2020b). These costs can involve reduced flight performance (Hedenström and Sunada, 1999), or the temporal loss of defensive armour (e.g. exoskeleton; Harvey, 1993), resulting in increased vulnerability and predation risk (Lucas et al., 2000). Moreover, moulting individuals often undergo major physiological changes that significantly alter metabolic requirements (e.g. higher protein demand) and thermoregulatory costs (Lindström et al., 1998; Murphy and Taruscio, 1995).

Birds provide an excellent system to study the evolutionary, ecological and physiological aspects of moult because the quality of their integuments (i.e. feathers) directly influences their survival and fitness (Dawson et al., 2000; Jenni and Winkler, 2020b; Serra et al., 2007). For example, in flying species, plucking a single flight feather may reduce flight performance and increase individual energetic costs, while some penguins are impaired in foraging and have to fast over weeks during their moult (Groscolas and Cherel, 1992; Hedenström and Sunada, 1999). However, apart from locomotion and insulation, feathers and plumage ornaments also play a crucial role in species recognition and camouflage, they allow adults to be distinguished from immatures and males to be distinguished from females, and they underlie sexual selection (Groscolas and Cherel, 1992; Hill and McGraw, 2006). Therefore, the process of renewing feathers gives rise to strong selection processes and arguably plays an important role in shaping avian life history (Kiat and Sapir, 2018). Given the critical importance of feathers in a bird's life, one may expect that selection enforces plumage maintenance and renewal of the feathers in an impeccable state. To achieve this, all birds drop their worn feathers and regrow them regularly; for some species, even multiple times a year. As

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moulting represents a major life-history stage, its timing is tightly controlled to be optimal for the given species' environment and life history (Barta et al., 2008; De La Hera et al., 2009; Holmgren and Hedenström, 1995; Kiat and Sapir, 2017). Despite the strong evolutionary implications, we know remarkably little about the physiological regulation of moult in birds (Dawson, 2015; Jenni and Winkler, 2020b). Although several hormone families have been recognized to affect moult (e.g. glucocorticoids, prolactin, thyroid hormones), the role of one of the most fundamental growth-regulating hormones, insulin-like growth factor 1 (IGF-1), has been entirely disregarded.

IGF-1 is an evolutionarily conserved peptide hormone that exists in all metazoan animals (Chan and Steiner, 2000). It is the main ligand of the insulin/insulin-like growth factor signalling pathway that regulates metabolism, cell proliferation and survival in the majority of invertebrate and vertebrate taxa (Barbieri et al., 2003; Schwartz and Bronikowski, 2016). In vertebrates, IGF-1 is considered to hold an important role in regulating key life-history transitions and thus has been suggested as a major mediator of life-history decisions (Dantzer and Swanson, 2012). Among the many functions of IGF-1, one of the best documented is its effect on somatic growth. In fact, IGF-1 increases the rate of post-natal growth in fish (Reinecke et al., 2005; Wood et al., 2005), reptiles (Sparkman et al., 2010), mammals (Lewin et al., 2017; Swanson and Dantzer, 2014) and birds (Lodjak et al., 2014, 2017). In mammals, IGF-1 also induces the proliferation of hair follicles and inhibits apoptosis, and therefore helps to keep them longer in the active (so-called anagen) phase and delay their transition to the regressive (catagen) phase (Li et al., 2014; Weger and Schlake, 2005). Transgenic mice over-expressing IGF-1 in the skin show earlier hair follicle development than controls, albeit at the price of developing dermal abnormalities and spontaneous tumour formation (Bol et al., 1997). IGF-1 also increases the rate of hair growth in tissue cultures (Ahn et al., 2012). Consistent with the role of IGF-1 in hair formation, some medical conditions characterized by either an excess or a lack of IGF-1 levels are also associated with abnormal hair growth. For instance, patients with primary IGF-1 deficiency (known as Laron syndrome) show sparse hair growth, while women affected by hirsutism (extreme excess of facial hair), have unusually high IGF-1 levels (Trüeb, 2018).

While these results suggest a role for IGF-1 in the regulation of hair growth, these studies were mostly performed on human tissue cultures or in rodents. Studies of how IGF-1 affects moult in other vertebrates are extremely scarce, but when available, seem consistent with its role in the regulation of moult. For instance, naturally shedding garter snakes were found to have higher circulating IGF-1 levels than non-shedding snakes (Sparkman et al., 2009), while a recently published study indicated a positive relationship between plumage quality, feather vane length and IGF-1 in a passerine species (Mahr et al., 2020). However, to date, there is only one experiment focusing on the relationship between moulting and IGF-1 in birds, revealing that stress-induced moult was associated with an increase in plasma IGF-1 levels in the broiler chicken (Mazzuco et al., 2005). To the best of our knowledge, no study has ever tested experimentally whether systemic IGF-1 affects the growth of feathers during moult.

In this study, we experimentally manipulated circulating IGF-1 levels in a passerine species undergoing a natural moult and investigated whether an elevation of IGF-1 resulted in an increased growth rate of flight feathers, higher moult intensity (increasing the number of feathers replaced at once) and changes in the quality of feathers. We expected that a systemic signal of elevated IGF-1 levels during natural moulting would induce faster growth of the feathers and/or higher moult intensity.

MATERIALS AND METHODS

Study animals and general protocol

We studied bearded reedlings, *Panurus biarmicus* (Linnaeus 1758), a common wetland specialist Eurasian songbird. Between July and October, this species undergoes a complete post-juvenile moult to acquire the first adult plumage. During this time, birds moult intensively, by growing several primary feathers and often some or all of their tail feathers simultaneously, which reduces their flying ability (Pearson, 1975; Spitzer, 1972; Wawrzyniak and Sohns, 1986). Between 28 and 30 July 2017, we captured 41 juvenile bearded reedlings using mist nets at Hortobágy-Halastó (47°38′13.7N and 21°04′42.8E, Hungary). We ringed all birds using numbered metal rings and measured their body mass (to the nearest 0.1 g) and tarsus length (to the nearest 0.1 mm). We determined the moulting stage immediately after capture by examining growing feathers in the wing, tail and body plumage. Detailed quantification of the moulting stage was achieved by scoring the moult of the primary wing feathers and tail feathers on a scale of 0–5, using the standard protocol for recording the progress of feather growth as suggested by the British Trust for Ornithology and further described in Jenni and Winkler (2020a).

Individuals that had not yet started the moult were released at the field site, resulting in a total of 41 moulting juveniles that were transferred into the housing facilities at the University of Debrecen. The bearded reedling is a highly social species and individual housing might result in stress; therefore, we kept 4 birds with similar moulting scores in a single cage (measuring 100×30×50 cm L×W×H). The cages were placed in an outdoor aviary, so the birds were protected from rain but experienced natural daylight and temperature fluctuations. Food (a mixture of freshly grated carrots, apples, quark, hard-boiled eggs, cracked dried fish, a commercial soft food mixture for insectivorous birds and ground cat food as a protein supplement, live mealworms daily and occasionally small crickets, grasshoppers and immature Turkestan cockroaches) and water was provided *ad libitum*. Once the experiment was finished, all birds were released in good condition into two spacious outdoor aviaries. We followed all applicable international, national and institutional guidelines for the use of animals. The study was approved by the institutional animal care and use committee and the regional government agency (licence no HBI/01/2708/2015).

IGF-1 manipulation

We manipulated IGF-1 levels using an injection of poly-(lactic-co-glycolid acid) (PLGA) microspheres prepared by S.V.-K. and B.A.G. as described previously (Luginbuehl et al., 2013; Meinel et al., 2001). Briefly, microencapsulation of recombinant human IGF-I was performed by solvent extraction from a $W_1/O/W_2$ dispersion. For this, the internal aqueous phase (W_1), containing IGF-I, 10 mmol l^{-1} sodium succinate and 140 mmol l^{-1} sodium chloride (pH 6.0) with bovine serum albumin as stabilizer, was emulsified in a solution of PLGA in dichloromethane (O) by ultrasonication. This W_1/O dispersion was introduced into a 5% (w/v) aqueous PVA solution (W_2) to form, under mechanical stirring, a $W_1/O/W_2$ dispersion. For solvent extraction, the $W_1/O/W_2$ dispersion was subsequently diluted with de-ionized water and stirred with a magnetic stirrer. The resulting microspheres were collected on a regenerated cellulose (RC) membrane filter and dried under reduced pressure at room temperature overnight. The microspheres had a loading of 272 ng IGF-I mg^{-1} microspheres.

Journal of Experimental Biology (2021) 224, jeb242481. doi:10.1242/jeb.242481

The treated birds received a total of 600 ng IGF-1 per injection. We injected 100 µl of dispersion subcutaneously into the back of the birds, between the shoulders. We followed the same protocol for control birds, with the exception that they were injected with 100 µl of the dispersion medium only.

Immediately before the manipulation, the necessary amount of microsphere particles was measured on an analytical balance (±0.1 mg) and an aqueous dispersion medium (containing 1.5% carboxymethyl cellulose sodium, 5% mannitol, 0.02% polysorbate 80) was added. The dispersion was then vigorously vortexed for 30 s, during which time it became homogeneous. The dispersion was vortexed again before injection.

We used a randomized block design, where half of the birds in a cage were randomly allocated to the treatment, while the remaining two birds were used as controls. The treatments were staggered so that two blocks (i.e. 8 birds) were processed in a day.

Experimental protocol

Experiments started following an acclimation period of, on average, 2 weeks. On the day of the treatment (day 0), two experimenters entered the aviary and captured the birds in the assigned blocks and collected a blood sample (~75 µl) into heparinized capillary tubes by puncturing the brachial vein with a 26G needle. Assistants recorded the time when the experimenters entered the room, and when blood samples were collected for each bird. Variation in sampling time did not affect our conclusions. Samples were kept on ice until transferred to the lab (within 1 h). Samples were centrifuged for 10 min and the plasma was removed with a Hamilton syringe and stored at −20°C until hormone assay.

After blood sampling, we recorded the body mass of each bird, scored their moulting stage as described above and evaluated moult intensity by counting the number of primary and tail feathers growing simultaneously (Fig. 1). Moult index was calculated as the sum of the moulting scores of individual feathers (with left and right side averaged) (Vágási et al., 2010). We also measured the planar length of the growing primary and tail feather vanes. We measured the part of the feather that emerged from the sheath using a digital calliper (to the nearest 0.01 mm) and refer to these measurements as 'feather vane length' throughout the paper. For freshly growing feathers that had only a tubular part (i.e. sheath still closed), this value was 0. After the measurements, birds were injected with the IGF-1 or control dispersion and released back into their cage and left undisturbed for the rest of the day. On day 1 and day 4, blood sampling, body mass and growing feather vane length measurements were repeated as described above, but the moulting scores were not recorded, to minimize handling time and because it was not reasonable to expect changes in the moulting stage during this short time. Day 1 and day 4 blood samples and measurements were always taken at the same time of day as the first samples to avoid diel variation in the hormone levels. On day 15, birds were measured again, and this time the moult stage was also recorded. All measurements were taken by the same person, who was blind to the treatment of the individuals.

After the last measurement, birds were released back into their aviary and were kept on *ad libitum* food and water. Once all birds had completed their moult, we measured their wing and tail length (to the nearest mm) and, for males, the length of their beard with a calliper (to the nearest 0.01 mm). We also plucked the innermost longest tail feather (Ta1). The quality of Ta1 tail feathers was assessed by measuring four parameters: feather length, rachis diameter, feather mass and the number of fault bars (Dawson et al., 2000; Jovani and Rohwer, 2017; Pap et al., 2008). Feather length was measured with a ruler (±0.5 mm) as the distance between the base of the calamus and the tip of the vane. The rachis diameter was measured across the dorsoventral plane with a digital calliper to the nearest 0.01 mm at the base of the vane (superior umbilicus). Tail feather mass was measured with a digital analytical balance (Axis AGN200, accuracy class I, e=0.001 g; d=0.0001 g). Fault bars are narrow malformations in feathers that appear as translucent bands oriented almost perpendicular to the rachis, where the feather vane and even the rachis may break (Jovani and Rohwer, 2017).

Statistical analyses

Statistical analyses were performed in R version 3.6.3 (http://www.R-project.org/). The effectiveness of the treatment was analysed in a linear mixed model, with log-transformed IGF-1 levels as the dependent variable, sex, treatment and days since the onset of treatment as a factor, the treatment×day interaction as fixed factors and individual identity as a random intercept. All these factors were significant, so further model reduction was not possible. Because the treatment×day interaction was significant, we calculated predicted marginal means and compared the IGF-1 and control group within each treatment day, and reported these results. These pairwise comparisons were implemented using the function pairs in the package emmeans, and *P*-values were adjusted using the Tukey HSD method.

Feather growth was analysed as the daily growth rate of feathers. This value was obtained by fitting a linear model to each individual feather with feather vane length as the dependent variable and day (0, 1, 4) as a linear independent variable, and extracting the slope from these models. Different statistical approaches provided very

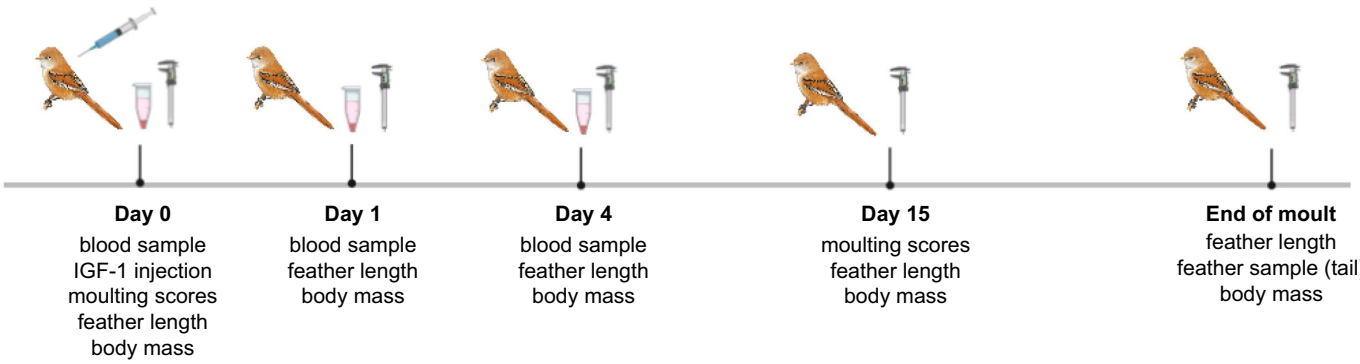

Fig. 1. Schematic representation of the experimental protocol. The syringe indicates the insulin-like growth factor 1 (IGF-1) manipulation; the Eppendorf tube and calliper symbolize the events of blood sampling and measurements, respectively.

Journal of Experimental Biology (2021) 224, jeb242481. doi:10.1242/jeb.242481

similar results. Feathers grew at a steady rate (see Results) so linear models had a good fit to the data (average adjusted R^2=0.93); thus, these estimates had very little uncertainty. When a feather was not yet visible on day 0 and day 1, the 0 value from day 0 was excluded before fitting the linear model. Growth rate was analysed in linear mixed models with Gaussian error distribution and with individual identity as a random effect. Moult intensity (the number of feathers growing simultaneously) and the number of fault bars were analysed with a Poisson distribution. For variables analysed with the Poisson distribution, we report z-values for statistical results, whereas for all other variables, we report t-values.

RESULTS

Before treatment

On day 0 of the experiment, all birds were moulting at least one inner primary feather, and many of them were also moulting their tail feathers (Fig. 2); 49% of the birds were also moulting parts of their flank feathers, 41% were moulting parts of their back feathers and only 5% started moulting the feathers on the head. The number of primary and tail feathers moulting was not different between sexes (z=1.639, P=0.101) and was not related to baseline IGF-1 levels (z=−0.427, P=0.669), but was positively related to body mass at the beginning of the experiment (z=2.260, P=0.023). On day 0, we found no difference between the treatment groups in any measure, including body mass, IGF-1 levels, moulting stage and length of growing feathers (all P>0.5).

Treatment effects

The treatment increased circulating IGF-1 levels the day following microsphere injection (day 1, P<0.001), but this difference disappeared by day 4 (P=0.809; Fig. 3).

Feather growth rate, moult intensity and feather quality

In the short term (over the first 4 days), primary feathers grew faster than tail feathers (t=−2.845, P=0.004), but IGF-1 treatment did not affect the growth rate of either primary or tail feathers (t=0.456, P=0.651, Fig. 4).

In the longer term, IGF-1 treatment altered the intensity of moult: 2 weeks after treatment, control birds were moulting fewer feathers than at the start of the experiment, while IGF-1-treated birds showed the opposite pattern and increased the number of feathers being moulted simultaneously, albeit with substantial individual variation (z=−2.064, P=0.039; Fig. 5A). As a result, 2 weeks after treatment, IGF-1-treated birds were in a more advanced stage of moult than controls (t=2.205, P=0.039; Fig. 5B). Once moult was completed, the final wing length and tail length did not differ between the treatment groups (wing: t=−0.33, P=0.741, tail: t=−0.321, P=0.750). The longest tail feather collected did not differ between the treatment groups in terms of feather mass (t=−0.94, P=0.354) or rachis diameter (t=−0.581, P=0.565). However, IGF-1-treated birds had fewer fault bars than control birds (z=−3.10, P=0.001; Fig. 6), indicating greater feather quality.

DISCUSSION

Our work is the first to explore the potential role of IGF-1 in regulating somatic growth events outside the postnatal period in a wild bird species, by examining its effects on feather moult. We conducted an exogenous hormone manipulation in wild-caught juvenile bearded reedlings and subsequently recorded moulting patterns and feather development in captivity. Our primary goal was to test whether an elevation of systemic IGF-1 affects the growth of feathers at the time when these feathers are growing during natural moult. The results of our experiments indicate that elevated IGF-1 levels were not related to feather growth rate. However, IGF-1 administration altered the intensity of moult: 2 weeks after the onset of the experimental procedure, birds that received IGF-1 treatment displayed a higher number of actively moulting primary and tail feathers than control birds and were at a more advanced stage of moult. Interestingly, IGF-1 did not affect feather length, mass and rachis diameter, but IGF-1 elevation affected feather quality, reflected in a lower number of fault bars on tail feathers of individuals that underwent hormone manipulation.

Based on previous research on the effects of IGF-1 on integument growth, renewal/replacement and development, we expected that

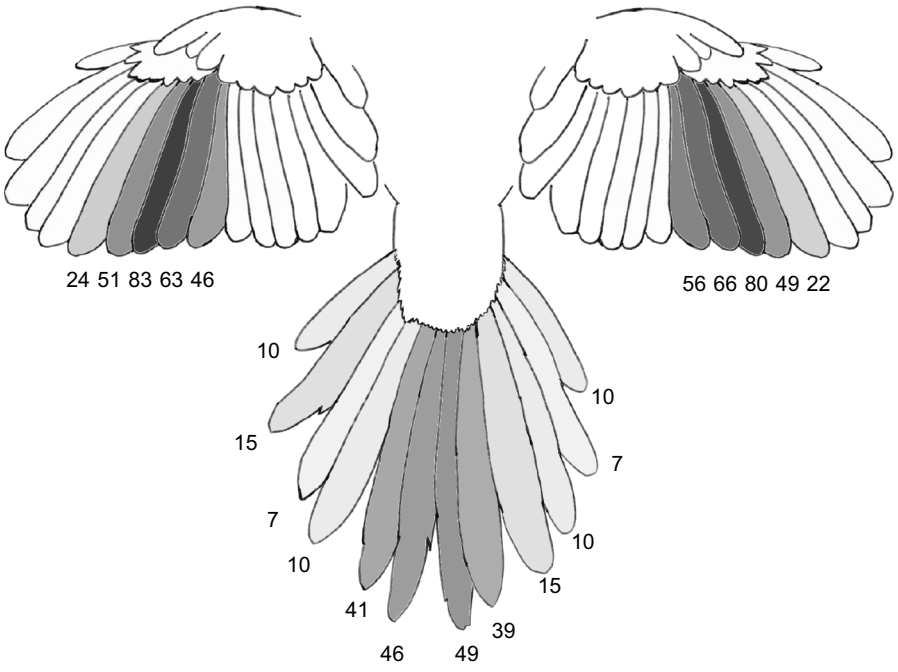

Fig. 2. Moulting status of juvenile bearded reedlings, *Panurus biarmicus*. Data were obtained at the start of the experiment (day 0, n=41 birds), prior to treatment. Shading is proportional to the percentage of birds moulting the given feather, which is also indicated numerically.

Journal of Experimental Biology (2021) 224, jeb242481. doi:10.1242/jeb.242481

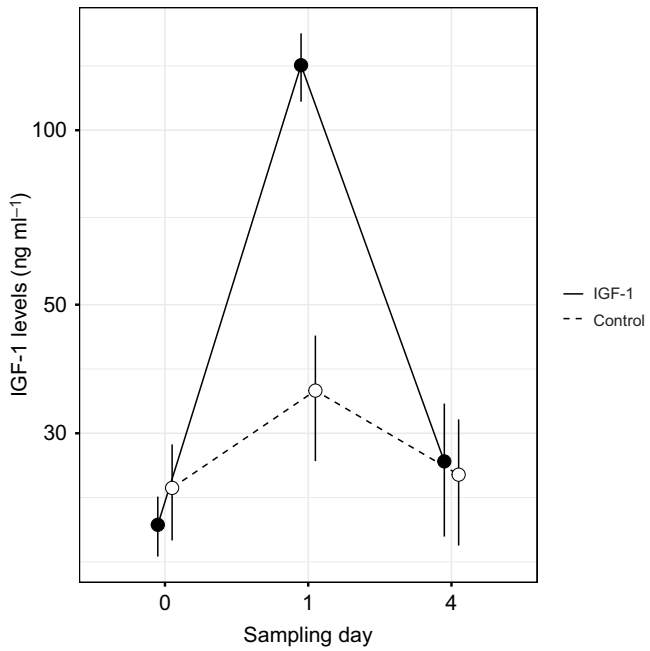

Fig. 3. Microsphere injection of IGF-1. IGF-1-injected birds showed increases in circulating IGF-1 levels relative to controls that lasted at least 24 h, but returned to baseline levels 4 days after the treatment (means±s.e.m.). IGF-1 levels are shown on a logarithmic scale.

IGF-1 supplementation would prolong and accelerate feather growth, resulting in longer and faster growing wing and tail feathers (Li et al., 2014; Weger and Schlake, 2005). We did not find this effect, even though natural variation in tail feather length has previously been shown to be associated with baseline IGF-1 levels in this species (Mahr et al., 2020). We consider the IGF-1 manipulation successful because a single injection of PLGA-microspheres elevated IGF-1 levels significantly above control on day 1 post-treatment (Fig. 3). While the treatment caused a pronounced elevation in IGF-1 levels on day 1, the peak hormone concentrations remained within the physiological range and were close to the highest concentrations found in naturally moulting individuals (Mahr et al., 2020). Our next sampling point was on day 4, by which time IGF-1 levels had returned to the baseline (and control) levels. Although the study design did not allow us to reveal the exact time point of the decline, follow-up investigations on the same species and method showed that significant treatment effects last up to 3 days and after an initial regression a second wave of release may last up to 7 days (K.M., Franz Gabor and Á.Z.L, unpublished results). These patterns indicate an exposure to elevated IGF-1 levels for at least 24 h in the current study and potentially longer, which is considerably longer than the average half-life (32 min, regardless of the dose) of simple IGF-1 injections used in previous studies (McGuinness and Cogburn, 1991), and highlights the usefulness of slow-release, biodegradable microspheres as a promising drug delivery system to manipulate hormones with minimally invasive administration. Despite this clear surge in IGF-1 levels, the growth rate of the primary and tail feathers remained very similar between the control and treatment groups between day 0 and day 1, and after that until day 4 (Fig. 4). Although we are not aware of any comparable study on feather growth, our results contradict some previous experiments in mice, showing that IGF-1 over-expression is related to exaggerated hair growth (Bol et al., 1997). In contrast to hair growth, the growth of primary wing feathers might be more tightly regulated, because they play a crucial role in flight efficiency. From that perspective, an insensitivity towards variation of systemic IGF-1 levels may be adaptive. IGF-1 may exert its effect locally by autocrine or paracrine regulation (Su et al., 1999), and birds may benefit from disentangling the control of feather growth from fluctuation of endocrine source of IGF-1. For example, stressors may cause a decrease in circulating IGF-1 levels (Tóth et al., 2018), and it may not be beneficial if that would affect the growth rate or the size of the developing flight feathers. The fact that the growth of the tail feathers was also unaffected by the manipulation is more surprising as they are sexually selected ornaments in this species (Romero-

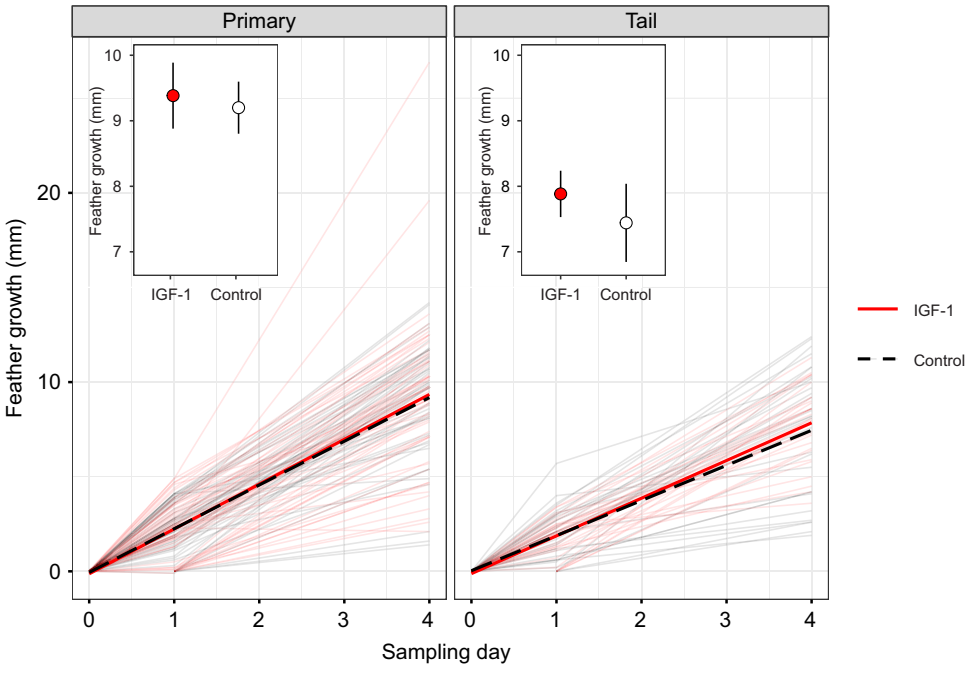

Fig. 4. Effect of IGF-1 on feather growth rate. The growth rate of primary and tail feathers did not differ between IGF-1-treated and control birds, but primary feathers grew faster than tail feathers. Note that the vane of some feathers emerged only on day 1. The thick lines represent the average growth per treatment, while the thin lines show the growth pattern of individual feathers. Values on the *y*-axis show the actual feather vane length minus the feather vane length on day 0, i.e. the amount of feather grown. The insets show feather growth (means±s.e.m.) during the 4 days per treatment.

Journal of Experimental Biology (2021) 224, jeb242481. doi:10.1242/jeb.242481

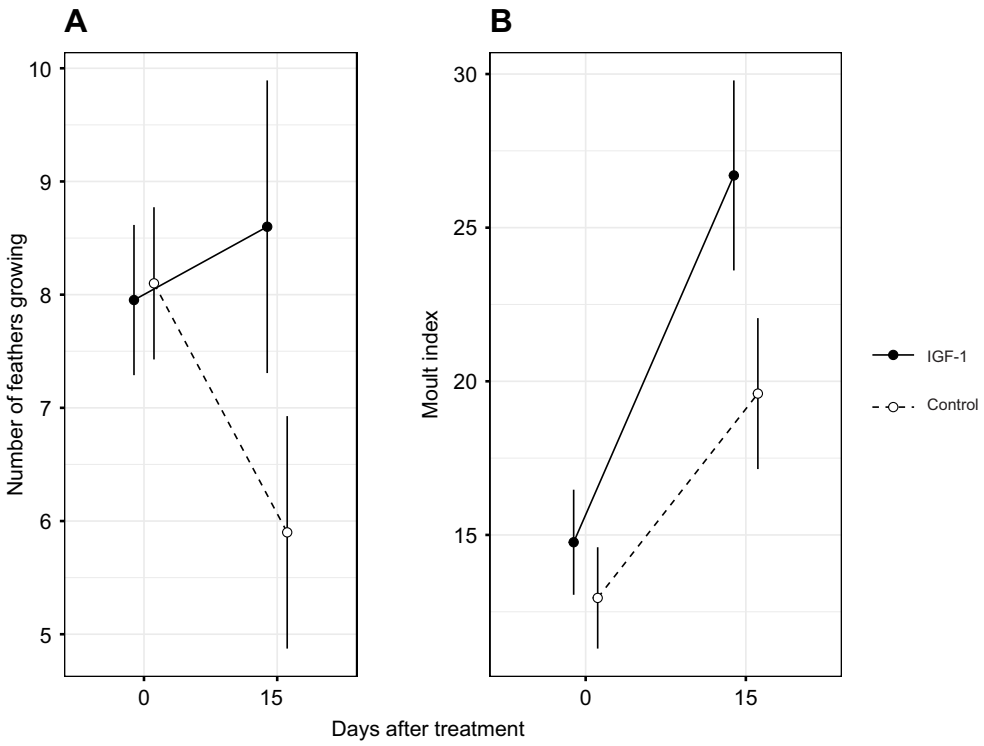

Fig. 5. Effect of IGF-1 on moulting. (A) Moult intensity (means±s.e.m.), measured as the number of simultaneously growing primary and tail feathers, changes in the opposite direction in birds treated with IGF-1 and controls. (B) As a consequence, 2 weeks after treatment, IGF-1-treated birds were at a more advanced stage of moult compared with controls.

Pujante et al., 2002) and natural variation in their length has been shown to be associated with baseline IGF-1 levels (Mahr et al., 2020). Sexually selected signals have been suggested to be related to insulin signalling as an assurance of their honesty (Warren et al., 2013).

Despite the robust lack of effect on feather growth speed, IGF-1 treatment maintained or even increased the number of feathers moulted simultaneously within 2 weeks, while the number of simultaneously growing feathers dropped in controls within the same period (Fig. 5). These results resemble the experiment by Li et al. (2014), who demonstrated that IGF-1 manipulation in wild-type mice stimulated cell growth and led to an increase in the number of hair follicles growing. This is further supported by *in vitro* studies, which have shown that IGF-1 increases the rate of hair growth in tissue cultures and is positively related to early follicle development (Ahn et al., 2012). In contrast to hair, the number of feather follicles that produce wing and tail feathers is small and shows no variation within species (~35 hair follicles mm^{-2} in mice versus the fixed number of 20 primaries and 12 rectrices). Furthermore, the moult of avian species often follows particular strictly controlled species-specific patterns, because gaps in the wing and tail feathers can greatly affect locomotion, with serious effects on individual fitness by increasing energy expenditure and vulnerability towards predators (Swaddle and Witter, 1997). Some species, however, moult several wing and tail feathers simultaneously, which also applies to the bearded reedling (Buker et al., 1975; Massi and Spina, 1996; Pearson, 1975). The question arises how IGF-1 might affect moulting patterns and whether elevated IGF-1 levels can serve as an adaptive function to facilitate faster moulting under specific environmental conditions. In mice, IGF-1 supplementation led to a premature transition of hair follicles to the anagen phase and protected them from turning into the catagen (regressive) phase (Ahn et al., 2012). These effects might also apply to feather growth in birds, facilitating a faster replacement of moulted feathers. While the primary feathers are replaced in a regular sequence in the bearded reedling, other body parts (especially the tail feathers) show a much less regular pattern and the moult may naturally decelerate in more advanced stages (Buker et al., 1975; Massi and Spina, 1996). Whether the decrease in the number of growing feathers in the control group was due to the natural moulting dynamics in this species or was an artefact of the captive conditions remains unknown; the important observation is that the same effect was not observed in the IGF-1-treated

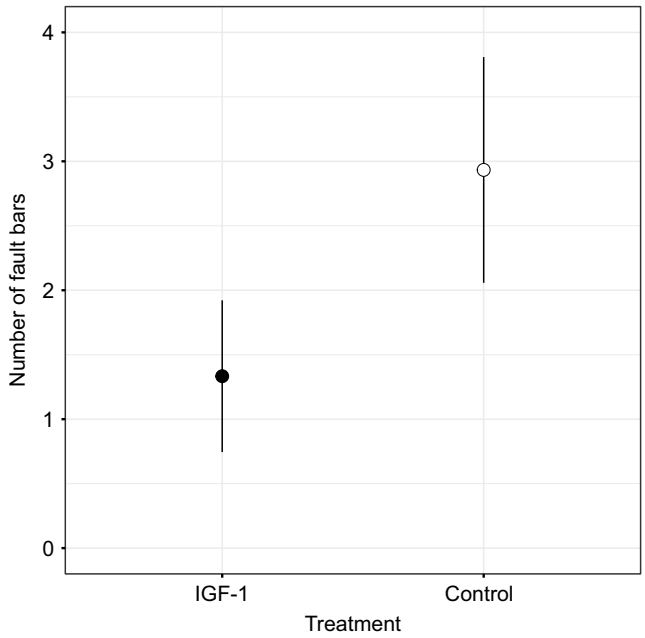

Fig. 6. Effect of IGF-1 on feather quality. At the end of the moult, IGF-1-treated birds had fewer fault bars in their longest tail feather than control individuals (means±s.e.m.).

group. Even though our results indicate that elevated IGF-1 levels do not increase growth rate per se, the moulting pattern may be affected and thus the overall time to moult might decrease. Moulting is an important life-history stage with major metabolic changes and there is a strong indication that different stressors can affect the synchronization and pace of feather growth in some passerine species. These disruptive factors may affect moulting in different ways. In starlings, for example, dietary restriction delayed the replacement of plucked feathers, whereas stress, simulated via corticosterone implants, decreased growth rate (Strochlic and Romero, 2008). IGF-1 is an important regulator of life-history changes and it is highly sensitive to the nutritional status of an individual and to dietary components (e.g. proteins) (Fontana et al., 2008; Miura et al., 1992). These characteristics are tightly linked to its important role in regulating energy allocation into cell proliferation, growth and protein synthesis (Dantzer and Swanson, 2012). Considering the metabolic function of IGF-1, the question arises whether IGF-1 also regulates the allocation of energy resources, required for feather growth (Mazzuco et al., 2005). Protein synthesis in tissues, for example, is partly promoted by and regulated through IGF-1 and this might also be of importance during the growth of feathers, in particular considering the increased demand for proteins during moulting (Murphy and King, 1992). Similar to experimental food restriction or supplementation, IGF-1 supplementation might lead to metabolic changes at the cellular level. These changes might affect moult intensity, i.e. the number of feathers growing simultaneously, rather than growth rate. It has to be considered, though, that we did not control for an effect of the dietary regime on moulting. All birds received an *ad libitum* diet in captivity, and the IGF-1 supplementation might have enhanced the effect of the high nutrient availability on feather growth. Moreover, as our study was designed to measure remiges and rectrices, our conclusions are restricted to these large individual feathers. We cannot exclude the possibility that our hormonal treatment may have affected the development of small feathers covering the entire body, similar to rodents and humans, where IGF-1 increases the number of developing hair follicles (Castro et al., 2012; Li et al., 2014). Rapid regrowth of feathers may be advantageous, because it shortens the time of reduced plumage functionality (e.g. reduced flight capability, weakened insulative capacity and signalling functions), but fast moult does have severe costs, such as a decrease in feather quality (Dawson et al., 2000; Vágási et al., 2010). In our study, moult intensity increased in the IGF-1-treated birds, indicating an accelerated moult rate, but we did not detect any negative effects on parameters of feather quality such as rachis diameter or feather mass. However, our results imply that IGF-1 might affect some aspects of feather quality, because birds receiving IGF-1 treatment had significantly fewer fault bars than birds of the control group (Fig. 6). These findings support a recent study by Mahr et al. (2020), showing that IGF-1 levels were positively correlated with structural plumage colouration in male bearded reedlings. Fault bars (also known as stress bars) are malformations of the feather, resulting from disruptions of feather growth that are often associated with stress or disease (Jovani and Rohwer, 2017). Similarly, the growth and regularity of the nano-sized structures that underlie structural plumage colours also partly depend on parameters of individual physiological condition during moult, with body condition being of particular interest. Hence, the discussed role of IGF-1 in regulating growth, cell proliferation and differentiation in relation to the nutritional status of an individual might also contribute positively to maintain feather structure throughout growth. In mammals, IGF-1 is also known to antagonize the effects of molecules inducing apoptosis (Ahn et al., 2012); this protective effect might also explain the lower number of fault bars observed in the IGF-1 treatment group. This function might be of particular importance during stressful events, when corticosterone levels rise substantially, while IGF-1 levels tend to decrease, but show large individual variation in this response. The interaction of corticosterone and IGF-1 has been suggested to predict fitness in growing songbirds (Lodjak et al., 2016). Corticosterone levels are known to have a seriously detrimental effect on growing feathers (Almasi et al., 2012; DesRochers et al., 2009; Jenni-Eiermann et al., 2015; Vágási et al., 2018), and it would be interesting to investigate how variation in IGF-1 levels during environmental challenges may contribute to escaping the damaging effects of stress-induced corticosterone. It is important to note that our manipulation increased circulating IGF-1 levels for 1–3 days, which is shorter than the time needed for the formation of the full feathers, which were analysed for quality indicators. However, the IGF-1 treatment had consequences well past the 1–3 day time frame (moult intensity and quality of the fully grown tail feathers), indicating that the treatment initiated physiological changes that persisted even after the circulating IGF-1 dropped back to original levels, and/or that the microspheres continued to release IGF-1 after a transient drop around day 4 (K.M., Franz Gabor and Á.Z.L., unpublished results).

If elevated IGF-1 levels accelerate moulting while simultaneously facilitating the maintenance of high feather quality, one might expect strong selection pressure towards high circulating IGF-1 levels. However, a recent study also indicated that the acute increase in IGF-1 levels was also associated with an increase in oxidative damage (measured as the levels of malondialdehyde, a marker of cellular membrane peroxidation and a reactive toxic agent in itself) (Lendvai et al., 2020 preprint). These results were further corroborated by another study in pied flycatchers showing that IGF-1 injection induced a significant increase in antioxidant levels, potentially to fight against increased oxidative stress (Lodjak and Mägi, 2017). Finally, we also found a relationship between circulating IGF-1 levels and oxidative damage in house sparrows, indicating some generality in the association between high IGF-1 levels and oxidative stress (Vágási et al., 2020). These results illustrate IGF-1's antagonistic relationships with several vital processes and therefore underlie its role as a major proximate effector of life-history trade-offs (Dantzer and Swanson, 2012).

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: A.Z.L., Z.T., K.M.; Methodology: A.Z.L., G.O., S.V., B.A.G.; Formal analysis: A.Z.L.; Investigation: A.Z.L., Z.T., K.M., G.O.; Resources: S.V., B.A.G.; Data curation: A.Z.L.; Writing - original draft: A.Z.L.; Writing - review & editing: A.Z.L., Z.T., K.M., G.O., S.V., B.A.G.; Supervision: A.Z.L.; Project administration: A.Z.L., Z.T.; Funding acquisition: A.Z.L., K.M.

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