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LIST OF ORIGINAL PAPERS

I. Lodjak, J., Mägi, M., Tilgar, V., **2014**. Insulin-like growth factor 1 and growth rate in nestlings of a wild passerine bird. *Functional Ecology* **28**, 159–166.

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IV. Lodjak, J., Tilgar, V., Mägi, M., **2016**. Does the interaction between glucocorticoids and insulin-like growth factor 1 predict nestling fitness in a wild passerine? *General and Comparative Endocrinology* **225**, 149–154.

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Author's contribution to the papers ('*' denotes a contribution equal to the amount of 0.33, '**' denotes a contribution to the amount of 0.66, '***' denotes the contribution to the amount of 1.00).

	I	II	III	IV
Original idea	***	***	***	***
Study design	**	***	**	**
Data collection	**	**	**	**
Data analysis	***	***	***	***
Manuscript preparation	**	**	**	**

1. INTRODUCTION

In vertebrate animals, postnatal growth rate is an important life-history trait that can influence individual fitness through survival and lifetime reproductive success (Stearns 1992). There is much evidence that differences in environmental resources, such as food availability and quality, are among the principle selective forces shaping fitness-related traits like physiological condition, growth and survival of animals during early postnatal development (Martin 1987; Dantzer & Swanson 2012). A complex combination of various hormone signalling pathways offers an underlying physiological mechanism for this life-history variation. Central to this phenomenon are insulin-like growth factors (IGF) and glucocorticoids, that alter gene transcription and protein synthesis of various signalling pathways (Nanto-Salonen *et al.* 1993; Sapolsky, Romero & Munck 2000; Lupu *et al.* 2001; Dufty Jr, Clobert & Møller 2002; Stratikopoulos *et al.* 2008). Yet, we lack a good understanding of how IGF-1 and corticosterone (main glucocorticoid in birds) promote growth and development in free-living animals in different environmental conditions (e.g. in relation to food availability), either by themselves or in interaction with each other.

IGF-1 is a growth hormone (GH) dependent peptide, which is either secreted into the systemic circulation by the liver or synthesized locally in many tissues as an autocrine/paracrine factor (Lupu *et al.* 2001; Bondy & Cheng 2004; Stratikopoulos *et al.* 2008). The signalling pathway and regulation of IGF-1 is well characterized and conserved among vertebrates (Rajaram, Baylink & Mohan 1997; Hwa, Oh & Rosenfeld 1999; Nakae, Kido & Accili 2001). The IGF signalling system includes three peptides (IGF-1, IGF-2 and insulin) as ligands, IGF binding proteins (e.g. IGFBP-1 – IGFBP-6 are described in mammals) and separate cell surface receptors for each ligand (Hwa, Oh & Rosenfeld 1999; Nakae, Kido & Accili 2001). However, among vertebrates, some differences in IGF signalling have been described; for example, the IGF-2 receptors of birds and fish do not bind to IGF-2, and birds seem to lack IGFBP-6 (Bassas *et al.* 1988; Daza *et al.* 2011). It is also important to note that every receptor in the described system has its own distinct role, but their mediated functions can overlap; for example, the growth promoting effects of IGF-1 are also able to use insulin receptors, although with lower affinity (Nakae, Kido & Accili 2001).

The growth and development promoting effects of IGF-1 in vertebrates are widely recognized throughout the embryonic and postnatal periods. It has been shown that IGF-1 and IGFBPs are expressed in embryonic bone, muscle, connective and neural cells in a time and tissue-specific manner and that the pattern is similar for chicken and mice (Ralphs, Wylie & Hill 1990). Through the stimulation of cell growth, differentiation and survival, IGF-1 promotes muscle mass increase, stimulates the remodelling of bone tissue and enhances neural as well as cardiac functions (Ren, Samson & Sowers 1999; O'Kusky, Ye & D'Ercole 2000; Yakar *et al.* 2002; Castellano *et al.* 2009; Otto & Patel 2010;

Mohan & Kesavan 2012). In fish and mice, the inhibition of IGF1R-mediated signalling ($igf1r^{-/-}$) severely reduces the size of embryos, causes developmental arrest, and all such affected animals die quickly during the postnatal period (Liu *et al.* 1993; Schlueter *et al.* 2007). Studies on mice with a IGF-1 receptor heterozygous knockout ($igf1r^{+/-}$) mutation, have shown substantial prenatal growth retardation (at birth the body mass was ~65% of a wild type) and this difference was more profound postnatally with increasing age (total accumulated postnatal body mass was 30–35% of a wild type; Liu & LeRoith 1999; Liu, Yakar & Leroith 2000; Lupu *et al.* 2001; Stratikopoulos *et al.* 2008). The same pattern has also been described in domesticated birds. *In ovo* injections of IGF-1 have been shown to promote overall body mass increase during the pre- and postnatal periods (Kocamis *et al.* 1998; Liu *et al.* 2011; Wang *et al.* 2012). Furthermore, in chickens which were divergently selected for high and low postnatal growth rates, the faster growing strain was shown to have increased plasma IGF-1 levels and hepatic IGF-1 mRNA expression rates, when compared to the strain with the lower growth rate (Beccavin *et al.* 2001; Giachetto *et al.* 2004). However, while this accumulated evidence shows that IGF-1 is essential during growth and development, a direct correlation between the IGF-1 and postnatal body mass is often reported to be absent (Buyse & Decuypere 1999; Giachetto *et al.* 2004; Rahimi 2005).

Given that resource allocation trade-offs are mainly driven by selective investment into different physiological mechanisms (Flatt & Heyland 2011), it is a challenge for evolutionary ecologists to link individual variation in physiology to individual variation in life history traits. In this respect, hormones, such as glucocorticoids, are important, albeit poorly understood, mediators underlying the regulation of postnatal growth and other fitness correlates among free-living vertebrates (but see Heath & Dufty 1998; Bonier *et al.* 2009). Glucocorticoids are evolutionarily conserved end product hormones of the hypothalamic–pituitary–adrenal (HPA) axis (Schulkin 2011) and are shown to have a pleiotropic effect on postnatal development among different vertebrate taxa. At sustained low levels, glucocorticoids stimulate neurogenesis, muscle and bone tissue development and immune system activation presumably through mineralocorticoid receptors (Bellows, Aubin & Heersche 1987; Smith 1990; Sapolsky, Romero & Munck 2000; Belanto *et al.* 2010; Anacker *et al.* 2013; Mazziotti & Giustina 2013; Saaltink & Vreugdenhil 2014). More specifically, in medical literature it is relatively well characterized that glucocorticoids at lower plasma levels promote the gene expression pattern needed for normal osteoblast development (Bellows, Aubin & Heersche 1987) and increase dysferlin expression needed for myogenesis (Belanto *et al.* 2010). Allostatic overload of individuals is associated with sustained elevated levels of glucocorticoids (McEwen & Wingfield 2003), which has been shown to have detrimental effects on postnatal growth (Morici, Elsey & Lance 1997; Capellan & Nicieza 2007; Wada & Breuner 2008), immune function (Siegel 1980; Pickering 1984; McEwen *et al.* 1997; Martin 2009), neuronal cell numbers and cognitive abilities (Howard & Benjamins 1975; Sui, Sandi & Rose 1997;

Kitaysky *et al.* 2003), and reproductive performance (Zanette, Clinchy & Smith 2006; Angelier *et al.* 2010) in different vertebrate taxa. Chronically elevated levels of glucocorticoids are thought to inhibit various physiological processes by binding to glucocorticoid receptors, or indirectly, by inhibiting the physiological signalling of growth hormone, insulin-like growth factors and thyroid hormones (Sapolsky, Romero & Munck 2000; Robson *et al.* 2002; Canalis *et al.* 2007; Mazziotti & Giustina 2013). This is done to overcome environmental challenges and to invest more into short term survival (Sapolsky, Romero & Munck 2000).

An important mechanism that affects overall fitness of an individual vertebrate through selective resource allocation between different physiological functions involves the interaction between glucocorticoids and IGF-1. At relatively low plasma levels, glucocorticoids mediate the maturation of the GH/IGF axis during prenatal development in chickens (Bossis & Porter 2003; Zheng *et al.* 2008). Furthermore, it has been shown that glucocorticoids are needed for growth hormone synthesis, and when thyroid hormones are present, this physiological effect is even more pronounced (Martial *et al.* 1977; Mazziotti & Giustina 2013). On the other hand, when environmental challenges for animals increase, high glucocorticoid levels start to inhibit costly investment into the GH/IGF axis. For example, in the tilapia (*Oreochromis mossambicus*), administration of a relatively high dose of exogenous cortisol (main glucocorticoid in fish) significantly decreased plasma IGF-1 levels in the blood and IGF-1 mRNA expression in the liver (Kajimura *et al.* 2003), suggesting that a decrease in plasma IGF-1 levels is mediated through the attenuation of IGF-1 gene expression. This change, in turn, can be mediated by glucocorticoid-induced inhibition of growth hormone or its receptor synthesis as shown in humans and rats (McCarthy, Centrella & Canalis 1990; Unterman *et al.* 1993). Interestingly, to some extent IGF-1 can modulate the physiological effect of glucocorticoids, mainly through peripheral metabolism. For example, IGF-1 inhibits the expression of 11β-hydroxysteroid dehydrogenase 1 and enhances the expression of 11β-hydroxysteroid dehydrogenase 2 in the adipose tissue and liver, which means that the local conversion of glucocorticoids from an inactive to active form is decreased and the clearance rate of the hormone from the bloodstream is increased (Paulsen *et al.* 2006). It has also been shown that IGF-1 acts as a reparatory mechanism for tissue damage inflicted by sustained high glucocorticoid levels (Stitt *et al.* 2004; Latres *et al.* 2005; Pansters *et al.* 2013).

Furthermore, studies in humans have shown that nutritional conditions during early postnatal stages of development modulate the developmental rate of individuals through changing the levels of IGF-1 and glucocorticoids (Cianfarani *et al.* 1998). Primarily, this nutrition-mediated hormonal interaction likely functions via two physiological mechanisms. Firstly, the levels of adipocyte-derived leptin (anorexigenic hormone) and liver-derived ghrelin (orexigenic hormone) are sensitive to the nutritional condition of the organism and likely influence synthetic pathways for glucocorticoids and IGF-1 via the hypothalamus paraventricular nucleus. This likely occurs in a similar way in

mammals and birds (Inui 2001; Cassy *et al.* 2003; Li *et al.* 2011; Kaiya, Kangawa & Miyazato 2013). In addition, under food-limited conditions, an increased level of ghrelin, which increases the food intake of an individual (Wren *et al.* 2001), has a direct stimulatory effect on the secretion of GH (Takaya *et al.* 2000; Kaiya, Kangawa & Miyazato 2013). It is important to note that IGF-1 secretion in response to GH is likely initiated, only when the nutritional compounds are present. Secondly, the activation of the IGF-1 initiated growth promoting phosphatidylinositol-3 kinase/protein kinase-B/mammalian target of rapamycin (PI3K/AKT/TOR) signalling pathway needs additional signals from nutritional compounds (e.g. amino acid), whereas it is inhibited in conditions where food is a limiting factor (Fingar & Blenis 2004).

Arguably the determination of the level of plasma glucocorticoids alone may be insufficient to predict the degree to which these hormones influence long term ecological effects (Landys, Ramenofsky & Wingfield 2006). In recent years several avian studies have used a non-invasive corticosterone measure from feather samples, which incorporates an integral hormonal profile over a relatively long time period, during which the feather was growing (Bortolotti *et al.* 2008; Bortolotti *et al.* 2009; Lattin *et al.* 2011). For example, Fairhurst *et al.* (2013) showed that changes in body mass and wing length from day 7 to day 11 post-hatch were associated negatively with feather corticosterone levels in nestlings of the free-living tree swallow (*Tachycineta bicolor*). It has also been shown in captive rhinoceros auklets (*Cerorhinca moncerata*) that the level of feather corticosterone increases in response to food deprivation (Will *et al.* 2014), but decreases in captive Caspian tern (*Hydroprogne caspia*) chicks (Patterson *et al.* 2015). The direction of response may likely depend on how strongly nutritional deprivation affects development. However, the mechanisms of corticosterone deposition into feathers are not well known, and therefore caution should be maintained when connecting the level of feather corticosterone to baseline or acute plasma measures (Fairhurst *et al.* 2013; Patterson *et al.* 2015).

Environmental perturbations during early stages of development can affect offspring fitness through permanent changes in their stress response cascades (e.g. Boonstra 2013; Love, McGowan & Sheriff 2013). However, how chronic environmental stressors, such as food availability during early post-hatching development, affect the reactivity of the offspring HPA-axis and GH/IGF axis is still poorly understood. **Given that individual differences in corticosterone and IGF-1 levels likely influence potential resource allocation-dependent trade-offs, it is imperative to explore the developmental plasticity of HPA axis and GH/IGF axis functions in relation to environmental challenges. This was therefore the main aim of this thesis.** To do this, in the current thesis the growth conditions of great tit (*Parus major*) nestlings were changed by carrying out a brood size manipulation (**I**, **III**, **IV**), and an IGF-1-injection experiment was conducted with pied flycatcher (*Ficedula hypoleuca*) nestlings (**II**). The experiments were carried out to explore how changes in growth conditions influence plasma IGF-1 levels and corticosterone levels, as reflected by

an integrated hormonal measure – feather corticosterone levels. In addition, we studied how the changes in hormonal profiles subsequently relate to variability in offspring fitness-related traits, such as postnatal growth rate, physiological condition and fledging success. Specifically, the hypotheses tested were as follows:

1) Insulin-like growth factor 1 and fitness of nestlings (**I**, **II**).
   - Given that by manipulating brood size it has been shown that nestlings in reduced broods receive larger amounts of food and fledge with higher body mass than those in control broods (Tinbergen 1987; Smith 1990; Sanz & Tinbergen 1999; Pettifor, Perrins & McCleery 2001; Neuenschwander *et al.* 2003), it was predicted that increased energy intake should sustain higher IGF-1 secretion in altricial nestlings from reduced broods compared to those in control and enlarged broods (**I**). In return, higher IGF-1 levels should be a causal link mediating achievement of an increased growth rate and better body condition at the pre-fledging stage of nestlings (**I**, **II**).
   - Also, it has been shown previously that altricial nestling growth rate is highest in the middle of the fledging period (Tilgar & Mänd 2006). Therefore it was expected that plasma IGF-1 levels would be correspondingly higher in the middle of the fledging period to support more rapid development, and to decrease towards the end of the fledging period when the growth rate decreases (**I**, **II**).

2) Corticosterone and fitness of nestlings (**III**).
   - By manipulating brood size, it was expected that, compared to control broods, altricial nestlings from decreased broods should have a lower level of feather corticosterone (main avian glucocorticoid) and nestlings from enlarged broods should have a higher level. This relies on the fact that nestlings in reduced broods are growing in better conditions with a lower allostatic load (Tinbergen 1987; Pettifor, Perrins & McCleery 2001; Neuenschwander *et al.* 2003; Landys, Ramenofsky & Wingfield 2006).
   - Secondly, it was predicted that nestlings from decreased broods should have a higher growth rate than nestlings from control broods, and enlarged broods should have a lower growth rate than nestlings from control broods.
   - A sustained corticosterone level is positively associated with short term mobilization of energy stores for immediate use (e.g. for facilitation of begging behaviour), and negatively with costly physiological functions over a longer time period, such as those mediating body mass at fledging (Sapolsky, Romero & Munck 2000; Romero 2004). Therefore, it was expected that investment into feather corticosterone (potentially enhancing short term survival) is traded off with growth rate and body size at fledging.

- Further, given that fledgling survival is strongly dependent on fledgling mass (Naef-Daenzer, Widmer & Nuber 2001; Tarwater *et al.* 2010; Mitchell *et al.* 2011; Maness & Anderson 2013), it was also predicted that elevated feather corticosterone level is inversely related to fledging success of nestlings.
- Since the brood-rearing conditions (e.g. food availability) can vary between habitats in our study area from year to year (Mägi *et al.* 2009; Remmel, Tammaru & Mägi 2009; Sisask *et al.* 2010), it was expected that the magnitude of the negative relationships between feather corticosterone and offspring size, and survival at fledging is more pronounced in coniferous forests with respect to deciduous forests in good breeding years, while the situation is reversed in unfavourable years.

3) Interaction between insulin-like growth factor 1 and corticosterone and fitness of nestlings (**IV**).
- In wild passerines, IGF-1 levels measured from plasma (**I**) and corticosterone levels measured from feathers (**III**) are sensitive to growth conditions. Accordingly it was expected that this relationship is crucial for determining the plasticity of growth rate in great tits. The brood size manipulation was carried out with the great tit, to see how plasma IGF-1 and feather corticosterone are associated in nestlings during postnatal development under different nutritional conditions (Tinbergen 1987; Naef-Daenzer, Widmer & Nuber 2001; Pettifor, Perrins & McCleery 2001). Hypotheses rely on assumptions that the synthesis of corticosterone and IGF-1 are nutrition-dependent, and on the fact that their signalling pathways interact with each other at the molecular level (see above). More specifically, given that nestlings in decreased and enlarged broods are in relatively better and relatively worse conditions respectively, it was expected that the association between plasma IGF-1 and feather corticosterone differs between nestlings from those treatment groups.
- Secondly, it was expected that the nutrition-dependent inter-regulation of IGF-1 and corticosterone can be linked to short term fitness, in terms of fledging success, in passerine nestlings.

2. MATERIALS AND METHODS

2.1. Study system

Data were collected from May to June in the years 2012 (**I**, **III**, **IV**) and 2014 (**II**) from free-living great tit and pied flycatcher populations respectively near Kilingi-Nõmme (south-western Estonia; 58° 7′N, 25° 5′E). The study area (ca. 50 km^2) is covered by a mosaic of deciduous and coniferous forest patches, which are dominated by grey alder (*Alnus incana*) and silver birch (*Betula pendula*), and Scots pine (*Pinus sylvestris*) respectively. Nest-boxes (internal dimensions: 11 × 11 × 30 cm), mounted on tree trunks at a height of 1.5–1.8 m, were checked weekly to obtain data on the onset of egg-laying and clutch size and to predict theoretical hatching dates. At the end of the incubation period, the nests were monitored at least every second day to obtain hatching dates of the first egg (hatch date = day 0 post-hatch).

The great tit (Fig. 1A) is a small (ca. 19 g) insectivorous and socially monogamous passerine (*Passeriformes*). It is a cavity nesting bird, which in Estonia starts laying its eggs towards the end of April. The clutch of 8–12 eggs is incubated for 12–14 days, after which the nestlings fledge within 16–22 days.

The pied flycatcher (Fig. 1B) is a small (12–14 g) insectivorous, migratory and polygamous passerine. The pied flycatcher is a cavity nesting bird, which in Estonia starts laying its eggs in the middle of May. The clutch of 5–8 eggs is incubated for around 12–13 days, after which the nestlings fledge within 14–16 days.

Figure 1. Male great tit (*Parus major*) in panel A (Photo: Jaanis Lodjak). Female pied flycatcher (*Ficedula hypoleuca*) with nestlings in panel B (Photo: Marko Mägi).

2.2. Study design

2.2.1. Brood size manipulation

The brood size manipulation was carried out with 2-day old great tit nestlings for studies **I**, **III** and **IV**. Broods with the same hatching dates were randomly assigned to one of three treatment groups: decreased, control or enlarged broods. Two randomly selected nestlings from the decreased broods were relocated to corresponding nests of the enlarged group. Nestlings from the control group were handled in the same way, but they were returned to their own nests. Such a technique has been widely used to manipulate growth conditions of nestlings in great tits and other hole-nesting passerines (Hõrak *et al.* 1999; Pettifor, Perrins & McCleery 2001; Neuenschwander *et al.* 2003).

Body mass of great tit nestlings was measured to the nearest 0.1 g at 2 and 15 days old. Tarsus length was measured to the nearest 0.1 and 1.0 mm, respectively, on 15-day old nestlings. On day 15 post-hatch, two of their tail feathers (the outermost feather from each side) were taken from three randomly selected nestlings per brood, to measure corticosterone levels (**III**; **IV**). Blood samples were also obtained from the same nestlings when they were 8 and 15 days old, to measure IGF-1 levels (**I**; **IV**).

2.2.2. Hormone-injection experiment

The hormone-injection experiment was carried out with the pied flycatcher. Within every brood, at age 3 days post-hatch, four nestlings with the same hatching date were randomly assigned to one of two treatments: an IGF-1-injected group (two nestlings) and control group (two nestlings). All nestlings were individually marked during the course of the experiment with a non-toxic marker, coloured plastic rings and metal rings. During the treatment period, each nestling in the IGF-1-injected group and control group was subcutaneously injected near the keel of the sternum, with either an IGF-1 or control solution. Nestlings were injected a total of 7 times between days 4 and 11 post-hatch during the 13-day fledging period. Nestlings were not injected on days when a blood sample was obtained (day 7 and 13 post-hatch). As for studies in chickens, mice and humans (Mehls *et al.* 1993; Kocamis *et al.* 1998; Xu, Capito & Spector 2008), recombinant human IGF-1 was used as a hormonal medium. The hormonal concentration of the injected solution was similar to those used by studies with chickens (Huybrechts *et al.* 1992; Tixier-Boichard *et al.* 1992). The order in which the nestlings were treated in the nest each day and in which the nests themselves were treated within a day was randomized. During the course of the experiment, body mass (between days 3 and 13 post-hatch; to the nearest 0.1 g), tarsus length (between days 7 and 13 post-hatch; to the nearest 0.1 mm) and wing length (between days 8 and 13 post-hatch; to the nearest 1.0 mm) measurements of nestlings were obtained.

2.2.3. Hormone analysis

Plasma samples (20 μl) were subjected to acid–alcohol extraction and neutralized to working pH with TRIS. Total IGF-1 levels were analysed using an enzyme-linked immunoassay (ELISA). For our analysis, the assay was validated for great tits (**I**) and pied flycatchers (**II**) using the serial plasma dilution method. Samples were assayed in duplicates. For further details see **I**.

Feathers were cut into small pieces (< 5 mm^2) with scissors and diluted in 10 ml of methanol. Feather corticosterone was then measured using a radio-immunoassay (RIA) approach combined with the dextran-coated charcoal suspension method. All samples were analysed in duplicates. The hormonal analysis for great tits was validated with a serial dilution method. For further details see **III**.

2.2.4. Ethics of the experiments

The experiments that were carried out in this thesis comply with the current laws of Estonia, and were approved by the Animal Procedures Committee of the Estonian Ministry of Agriculture with the following licences:

- Licence no. 100 (17.01.2012)
  Granted permission to collect feather and blood samples from nestlings and adult birds of the great tit and pied flycatcher in the Kilingi-Nõmme study area. The study complied with the licence by not exceeding the number of individuals sampled from the given species and volume of the blood sample taken from nestlings.

- Licence no. 108 (02.04.2012)
  Granted permission to carry out the brood size manipulation with great tits from the wild population in the Kilingi-Nõmme study area. The study complied with the organizational conditions of the experiment, which were stated in the licence.

- Licence no. 31 (19.05.2014)
  Granted permission to carry out the IGF-1 injection study with nestlings of the pied flycatcher from the wild population in the Kilingi-Nõmme study area. The study complied with the licence by not exceeding the number of individuals used and the hormonal concentration injected. The number of injections made and types of samples collected also complied with the terms of the licence.

- Licence no. 1-4.1/11/100 (23.03.2011)
  Granted permission to disturb great tits and pied flycatchers during the nesting period to register their clutch size and brood sizes in the Kilingi-Nõmme study area. The licence also gave permission to collect feather and blood samples from great tits and pied flycatchers.

3. RESULTS AND DISCUSSION

3.1. Insulin-like growth factor 1 and fitness of nestlings (I, II)

Main results

1. The IGF-1 levels in free-living passerine nestlings are affected by growth conditions: nestlings in better growing conditions had higher levels of IGF-1, compared to those in worse growing conditions (**I**).

2. The IGF-1 levels changed with age: higher levels were in the middle of the fledging period. Interestingly, the pattern was similar for nestlings of the great tit (**I**) and pied flycatcher (**II**), when nestlings were 8 and 7 day olds respectively, compared to those at the pre-fledging stage, 15 and 13 days old respectively. IGF-1 levels decreased towards fledging, whereas the decrease was lower for nestlings in better growing conditions, compared to those in worse growing conditions (Fig. 2; **II**).

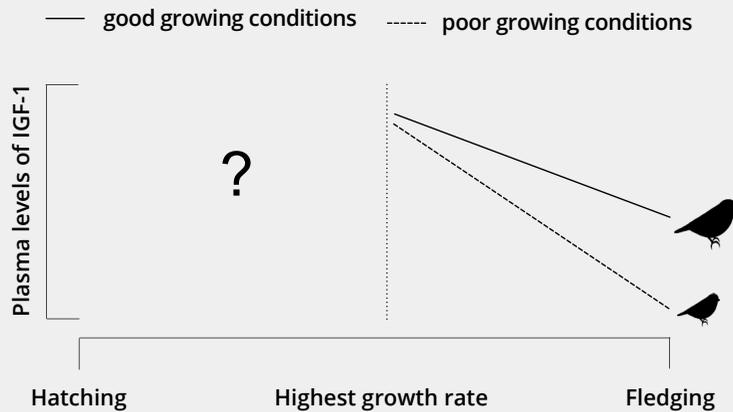——— good growing conditions ------ poor growing conditions

Figure 2. The change in plasma IGF-1 levels with age during early postnatal development of altricial nestlings. Levels of plasma IGF-1 were significantly higher in the middle of the fledging period (**I, II**). The decline in IGF-1 levels was greater in nestlings in poorer growth conditions (**II**). It is important to note that it is unknown how IGF-1 levels change with age prior to the midpoint of the fledging period ('?' in Fig. 2).

3. The proportion of IGF-1 invested into growth is higher for nestlings in better nutritional conditions. Interestingly, the proportion of IGF-1 invested into growth is more profoundly down-regulated towards the end of the fledging period for nestlings in better nutritional conditions, that reached the necessary body size sooner, compared to those in the worse nutritional conditions.
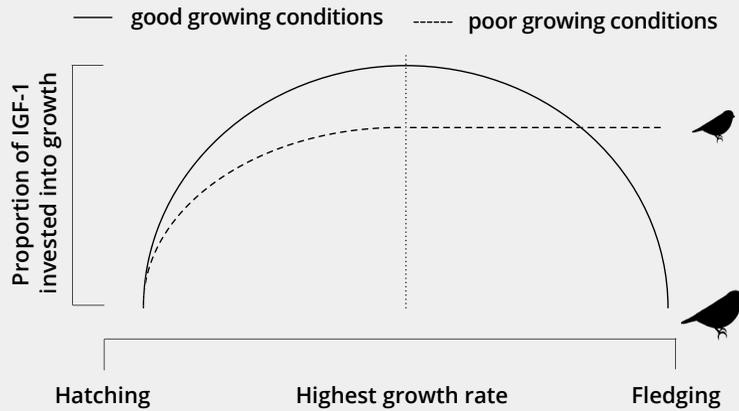

Figure 3. Relative change in IGF-1 investment into growth with age during early postnatal development in altricial nestlings. Relative change in IGF-1 investment into growth is higher in nestlings in the first half of the fledging period and is down-regulated towards the end of the fledging period in nestlings that grew in better conditions, as opposed to those in worse growing conditions.

The results of the current thesis show that the IGF-1 levels in nestlings of free-living passerines follow a gradient of resource (e.g. food) availability to mediate their postnatal growth rate (**I, II**). The variability in metabolic compounds (e.g. glucose, insulin, free fatty acids and proteins) has been shown to be associated with the changes in plasma IGF-1 levels (Ross & Buchanan 1990; Scacchi, Pincelli & Cavagnini 2003). With higher food availability, the increasing concentration of metabolic compounds in the bloodstream has rather mixed to suppressive effects on GH secretion, but dominantly up-regulatory effects on the synthesis of IGF-1 (Ho *et al.* 1988; Ross & Buchanan 1990; Beckman 2011; Mohan & Kesavan 2012). Conversely, when the availability of food for nestlings is limited, the decreasing concentration of metabolic compounds in the bloodstream tends to increase GH and decrease IGF-1 synthesis (Ross & Buchanan 1990; Breier 1999; Scacchi, Pincelli & Cavagnini 2003). Therefore, it could be said that somatic growth of passerines, and likely in other vertebrates, is largely a function of resource-dependent fluctuations in GH metabolic effects

and subsequent IGF-1 signalling (Ross & Buchanan 1990; Breier 1999; Scacchi, Pincelli & Cavagnini 2003). To further illustrate this, Lupu *et al.* (2001) showed in laboratory mice, that GH and IGF-1 explained 83% of the variability in asymptotic body mass, through their own respectively specific or functionally overlapping physiological effects. The results of the thesis are consistent with this; if nestlings received on average more food, the likely increased presence of nutritional compounds in the bloodstream of nestlings facilitated the maintenance of higher plasma IGF-1 levels and subsequent increased growth rate and body size when compared to nestlings that were raised in more nutritionally-restricted conditions (**I**, **II**).

It has previously been shown that plasma IGF-1 and hepatic IGF-1 mRNA expression increase gradually with age in the early postnatal development of precocial chickens (Beccavin *et al.* 2001; Giachetto *et al.* 2004). The results, in great tits (**I**) and pied flycatchers (**II**), show a somewhat different pattern to that described in chickens (Fig. 2). Plasma IGF-1 levels were higher in the middle of the fledging period (day 8 for great tits: **I**; day 7 for pied flycatchers: **II**) when compared to the pre-fledging period (day 15 for great tits: **I**; day 13 for pied flycatchers: **II**). It is important to emphasize that in this thesis there were only two age groups for each of the species, and no IGF-1 data were available for the early postnatal stage (**I, II**). Hence, the possibility cannot be excluded that IGF-1 levels were already in the declining phase in the middle of the nestling period (day 6–7 for great tits: Tilgar & Mänd 2006; day 5–6 for pied flycatchers: **II**). However, the great tit and pied flycatcher nestling growth rate is highest in the middle of the nesting period and decreases substantially towards fledging. This indicates that age-related changes in IGF-1 levels from the middle to the end of the fledging period follow the respective changes in somatic growth rate of nestlings in wild passerines.

The IGF-1 levels of the nestlings showed a strong negative correlation with growth rate and pre-fledging body mass (on day 15 post-hatch for great tits and day 13 post-hatch for pied flycatchers; Fig. 3) for great tit nestlings in reduced broods (**I**) and larger, more well-conditioned pied flycatcher nestlings (**II**), which is the opposite pattern to that shown in studies with poultry (Buyse & Decuypere 1999; Beccavin *et al.* 2001). No such pattern was found either in great tit nestlings from control or increased broods (**I**) and smaller, less well-conditioned pied flycatcher nestlings (**II**). The described pattern may be an indication of adaptive IGF-1 down-regulation in mature nestlings. To speculate, perhaps heavier, more mature chicks had a higher developmental speed and higher IGF-1 levels at the midpoint of the fledging period, or at least earlier than the pre-fledging stage, than smaller-sized, less mature nestlings. Therefore, fledglings that maintained high growth rates throughout development and reached their optimal fledging mass quicker, probably needed to invest less to somatic growth and structural size at the end of the fledging period. Hence, such nestlings could redirect energy from growth to other functions, thus allowing them to use more resources to cope with the environmental conditions experienced during the transition to independence (**I**, **II**).

3.2. Corticosterone and fitness of nestlings (III)

Main results

1. The level of corticosterone in free-living nestling feathers is affected by growth conditions in a habitat-specific way: nestlings in reduced broods had lower levels of feather corticosterone compared to those in enlarged broods only in coniferous forest.

2. The level of feather corticosterone was related to changes in nestling growth rate in a habitat-specific way. There was a polynomial association between feather corticosterone levels and growth rate only in nestlings from the coniferous forest, whereas the association between the traits was absent in nestlings from the deciduous forest.
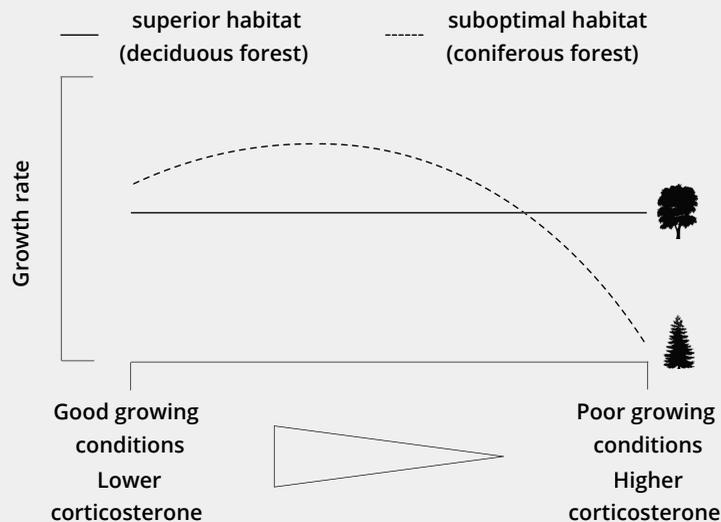

Figure 4. The association between feather corticosterone and growth rate in great tit nestlings in coniferous (dashed line) and deciduous habitat (solid line). The association was statistically significant only for coniferous forest.

3. The level of corticosterone in feathers of free-living nestling is affected by growth conditions and its level is related to changes in fledging success in a habitat-specific way: feather corticosterone levels were associated with fledging success in coniferous forest only, whereas the association was absent between the traits in deciduous forest.
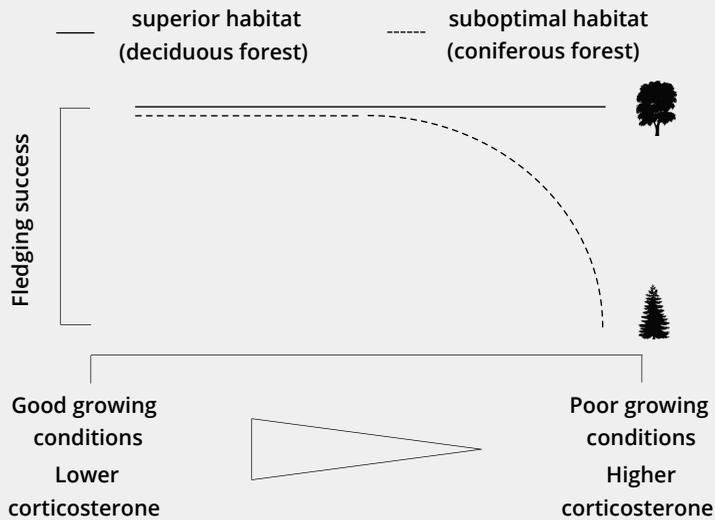

Figure 5. The association between fledging success and feather corticosterone of nestlings from enlarged broods in coniferous (dashed line) and deciduous habitat (solid line). The association was statistically significant only for enlarged broods from the coniferous forest.

The level of corticosterone in free-living nestling feathers is affected by growth conditions and its level is related to variability in nestling fitness, in terms of growth rate and fledging success. However, these relationships appear to be strongly habitat-specific and likely year-specific.

The results suggest that deciduous forests can be regarded as superior habitat for nestling growth during the breeding season in the study year 2012. Why? Firstly, fledging success declined with brood size only in the coniferous habitat. Possibly, parents in coniferous forests were not able to provide enough food for enlarged broods, probably due to limited availability of suitable food items. In good breeding years, great tits breeding in deciduous forests can produce more fledglings than those in coniferous forests (van Balen 1973; Newton 1998), although the situation can be reversed in bad years (Tilgar, Mänd & Mägi 2002; Mänd *et al.* 2005). Secondly, overall variability of feather corticosterone was significantly higher in the coniferous habitat in comparison with the deciduous

habitat, and feather corticosterone was increased in enlarged broods in the coniferous habitat only (**III**). This suggests that nestlings from such broods could be chronically stressed, probably owing to dietary restrictions coupled with increased sibling competition.

However, another question arises as to why pre-fledging body mass was not reflective of increased feather corticosterone or decreased fledging success in deciduous forest. Previous studies have shown that great tit nestlings attain full body size several days before fledging (Tilgar & Mänd 2006), while physiological processes related to maturation of other tissues, such as bone mineralization or wing growth, still last throughout the fledging time (Nilsson & Gårdmark 2001; Tilgar *et al.* 2008). Hence, it is possible that 15-day old great tit chicks from deciduous forest could be more developed than chicks in coniferous forest, but they are visually indistinguishable from each other prior to fledging. Moreover, the recession of nestling body mass and elevation of corticosterone prior to fledging (Heath 1997; Kern *et al.* 2001) may also explain why no habitat differences were found in pre-fledging body masses and corticosterone levels of great tit chicks. In altricial birds, conflict could occur over the timing of leaving the nest. Parents may benefit by shortening the nestling period and conserving energy for future reproductive attempts, moulting and/or migration. It is important to note that great tits are double breeders in our study area (Mägi & Mänd 2004) and the that sooner fledglings from the first brood leave the nest, the higher the chance for parents to have two successful broods during the same breeding season. Respectively, it has been shown in wild birds that parents reduce provisioning prior to fledging to encourage the chicks to leave the nest (e.g. Kern *et al.* 2001). Hence, these parental tactics may reduce nestling pre-fledging body mass and elevate their corticosterone levels, which can be important for initiating the process of fledging. In the case of the results obtained, the possibility cannot be excluded that nestlings in deciduous forests had reached the stage of body mass recession earlier than those in coniferous forests, or that nestlings in decreased broods exhibited more pronounced mass recession compared to those in enlarged broods in deciduous forests.

In this thesis, a curvilinear association between feather corticosterone levels and growth rate of great tit nestlings was shown (Fig. 4; **III**). This relationship is likely facilitated by the physiological, corticosterone concentration-dependent effect of two distinct receptors of the hormone (Schultner *et al.* 2013). Individuals in good nutritional condition are exposed to low corticosterone levels, likely mediated by mineralocorticoid receptors (Sapolsky, Romero & Munck 2000; Romero 2004). Its effects are dominantly anabolic and stimulatory on physiology and growth, thereby enhancing individual survival. When nestlings are in poor condition, corticosterone levels rise, and a greater number of corticosterone effects are thought to be mediated by glucocorticoid receptors, which tend to have more catabolic and inhibitory effects on various physiological functions, such as muscle mass development and bone formation (Sapolsky, Romero & Munck 2000; Romero 2004). Consistently, data of the current thesis showed a sustained high plasma corticosterone level inhibited

resource-demanding growth in order to enhance bodily functions related into immediate survival (e.g. energy mobilization, increased begging behaviour; Sapolsky, Romero & Munck 2000; Kitaysky *et al.* 2003; Loiseau *et al.* 2008). Hence, given that decreased growth rate was associated with lower fledging success, it seems that under poor growth conditions, larger physiological invest-ment to immediate survival might be traded off with longer term survival at the start of independence and later in life. Still, the described relationship between corticosterone levels and fitness correlates was only found in the coniferous forest (Fig. 5; **IV**). This may be explained by a lower variance of feather corti-costerone levels in deciduous forests, which would indicate that corticosterone signalling through the dual receptor system was more pronounced in the more variable coniferous habitat than in the deciduous habitat.

3.3. Interaction between insulin-like growth factor 1 and corticosterone and fitness of nestlings (IV)

Main results

1. The signalling pathways of IGF-1 and corticosterone may interact with each other to modulate the early postnatal development in nestlings of a wild passerine: pre-fledging plasma IGF-1 levels of high-quality, nestlings from decreased broods was positively associated with levels of feather corticosterone. On the other hand, the association between corticosterone and IGF-1 was negative in nestlings from enlarged broods.
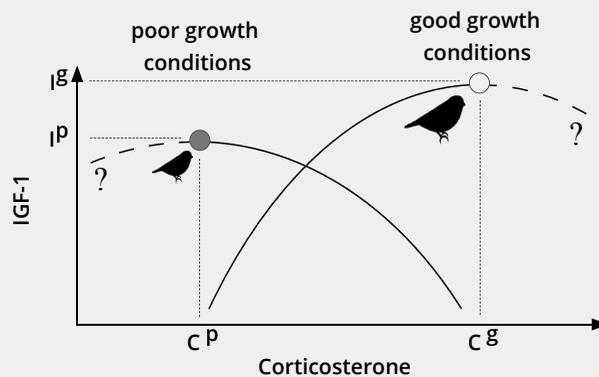

Figure 6. Schematic figure showing the theoretical relationships between IGF-1 and corticosterone in nestlings from decreased (good growth conditions) and enlarged (poor growth conditions) broods. C_g and C_p denote respectively the threshold levels of corticosterone in good and poor growth conditions at the inflection point, where its physiological effects tend to switch from being dominantly stimulatory to inhibitory on the synthesis of IGF-1. I_g and I_p denote respectively the levels of IGF-1 at the mentioned inflection point in good and poor growth conditions.

2. Fledging success is significantly predicted by the interaction between levels of IGF-1 and feather corticosterone: when nestlings had lower pre-fledging plasma IGF-1 levels, the corticosterone levels were negatively associated with fledging success. When pre-fledging plasma IGF-1 levels increased, the association between corticosterone levels and fledging success became positive.
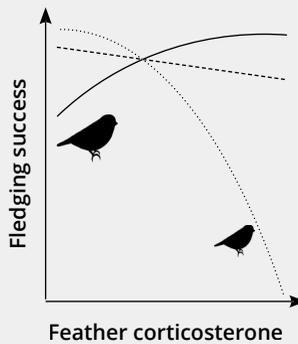
Figure 7. The association between fledging success and the continuous-continuous interaction between levels of feather corticosterone and plasma IGF-1 at the pre-fledging stage. IGF-1 levels for plotting purposes are divided into 3 quantiles. The dotted line denotes nestlings in the first quantile, the dashed line denotes nestlings in the second quantile and the solid line denotes nestlings in the third quantile.

IGF-1 levels were elevated in nestlings in good growing conditions compared to those in poor ones (**I**), while feather corticosterone level had a tendency to be lower in nestlings in good growth conditions (**III**). In this thesis, it was shown that the shape of the relationship between these two hormones is also nutrition-dependent. The pre-fledging plasma IGF-1 level of high-quality nestlings (higher growth rate, better physiological condition; **I**) from decreased broods was positively associated with levels of feather corticosterone (Fig. 6; **IV**). On the other hand, the association between corticosterone and IGF-1 was negative in nestlings from enlarged broods (Fig. 6; **IV**), whereas the correlation between the two hormonal measures was absent in nestlings from control broods (**IV**).

The emergence of a positive association between these two hormones in good growing conditions is likely facilitated by two complementary mechanisms. First, in good growth conditions, as opposed to poor ones, there seems to be no physiological trade-off between the organism's maintenance activities that are mediated by corticosterone levels, and somatic growth that is mediated by IGF-1 levels. Notably, this tends to be the universal pattern in various life-history trade-offs if the limiting effect of food is absent (reviewed in Zera & Harshman 2001). Second, the correlation between the levels of IGF-1 and corticosterone in decreased broods may likely be dependent on the degree of maturation of nestlings at the pre-fledging stage. Larger nestlings in decreased broods have a tendency to be more mature at the pre-fledging stage, they have almost completed their growth and they have no need to invest as much into growth through IGF-1 compared to smaller ones (**I**, **II**). Consequently, it could

be expected that more mature chicks in decreased broods have both low corticosterone and low IGF-1 levels. Less mature nestlings in decreased broods are likely still in the active growth phase, and they invest energy into growth (via increased IGF-1 synthesis) as well as into survival-related behaviours (increased corticosterone) without nutritional limitations. In this respect, increased corticosterone levels may provide nestlings with an immediately available energy resource and nutritional compounds via lipolytic activity and by maintaining necessary plasma glucose levels (Morgan *et al.* 2013; Kuo *et al.* 2015). High levels of IGF-1 also enhance the tissue-specific local conversion of glucocorticoids to their inactive form, thus firstly buffering against the detrimental effects of high glucocorticoid levels, and secondly, repairing glucocorticoid-dependent tissue atrophies (Stitt *et al.* 2004; Latres *et al.* 2005; Paulsen *et al.* 2006; Pansters *et al.* 2013).

On the other hand, in unfavourable growth conditions, 15-day old nestlings of enlarged broods are supposedly in the active growth phase. Due to energetic restrictions, these poor-quality nestlings (lower growth rate, worse physiological condition; **I**) are subjected to a selective allocation of resources between physiological functions, such as maintenance activities and somatic growth. Under these circumstances nestlings probably divert more energy to survival-related behaviours, such as increased begging in order to compete with other siblings for limited resources, at the expense of costly growth and development (Kitaysky, Wingfield & Piatt 2001; **III**). Consistent with this idea, it was found that nestlings in enlarged broods, which are growing in a more nutritionally-limited environment, exhibited a negative relationship between corticosterone and IGF-1 levels. In agreement with the previous finding, it has been shown in chickens and humans that under nutrient-limited conditions, high levels of glucocorticoids inhibit the synthesis of GH and IGF-1 (Bossis & Porter 2003; Zheng *et al.* 2008; Mazziotti & Giustina 2013) as well as the expression of their respective receptors (Jux *et al.* 1998; Klaus *et al.* 2000).

Given the discussion above, the hypothesis might be posed that threshold levels of corticosterone, over which its physiological effects switch from stimulatory to inhibitory on the activity of the GH/IGF axis, depend on growth conditions. In good conditions, the inflection point of the curve, reflecting the physiological redirection of internal energy sources from development to short term survival, is potentially achieved with relatively higher levels of corticosterone ($C_g > C_p$ in Fig. 6; **IV**) and IGF-1 ($I_g > I_p$ in Fig. 6; **IV**) compared to nestlings in poor conditions. It should be still mentioned that the exact shape of the association between corticosterone and IGF-1 towards values of corticosterone that are lower and higher relative to the inflection point, respectively for nestlings that grew in poor and good conditions is not yet known (Fig. 6; **IV**).

Nutrition is among the main drivers behind the fluctuations in plasma levels of IGF-1 and corticosterone. Since the hormones interact with each other at the levels of synthesis and down-stream signalling, it could be expected that the multilevel correlated regulation of these hormones acts as an adaptive physiological mechanism maintaining homeostasis of nestlings, therefore connected to

their fitness during the time in the nest. Consistently with this, it was shown in this thesis that fledging success was predicted by the interaction between the pre-fledging levels of plasma IGF-1 and feather corticosterone (Fig. 7; **IV**). In nestlings that had relatively lower levels of IGF-1 (in enlarged broods; **I**), levels of corticosterone were negatively associated with fledging success (Fig. 7; **IV**). In contrast to this, when the levels of IGF-1 increased (in decreased broods; **I**), the association between the levels of corticosterone and fledging success of nestlings became positive (Fig. 7; **IV**). Interestingly, contradictory patterns show that corticosterone is connected to survival during early postnatal development (e.g. Blas *et al.* 2007; Goutte *et al.* 2010; Rivers *et al.* 2012). Rivers *et al.* (2012) suggested that the mixed results for the survival–corticosterone association probably exists because the experimentally created environmental challenges for individuals varies greatly among the studies. This means that the association between the levels of corticosterone and a nestling's survival could change from negative to positive as the physiological demand from environmental pressures decreases. The results of the current thesis support this hypothesis and offer a likely mechanism for it. Nestlings from enlarged broods exhibit a retarded growth rate because they are not able to invest as much into IGF-1 mediated metabolism as those from decreased broods (Lupu *et al.* 2001; Sjögren *et al.* 2001; **I**). Secondly, lower IGF-1 levels likely cannot buffer the negative effects associated with increasing levels of corticosterone (Stitt *et al.* 2004; Latres *et al.* 2005; Paulsen *et al.* 2006; Pansters *et al.* 2013), which in turn are needed for nestlings to cope with changes in the environment, and sibling competition (Sapolsky, Romero & Munck 2000; Kitaysky, Wingfield & Piatt 2001; **III**). Therefore, the inverse relationship between levels of corticosterone and fledging success is expected to become stronger alongside the decrease in IGF-1 levels. On the other hand, nestlings from decreased broods grow up in good growth conditions and they are able to invest more into IGF-1 mediated metabolism and build-up of their body faster than nestlings from control and enlarged broods (Lupu *et al.* 2001; Sjögren *et al.* 2001; **I**). Furthermore, elevated IGF-1 levels can act as a more efficient buffer against the adverse physiological stress associated with increasing levels of corticosterone (Stitt *et al.* 2004; Latres *et al.* 2005; Paulsen *et al.* 2006; Pansters *et al.* 2013) promoting their short term survival.
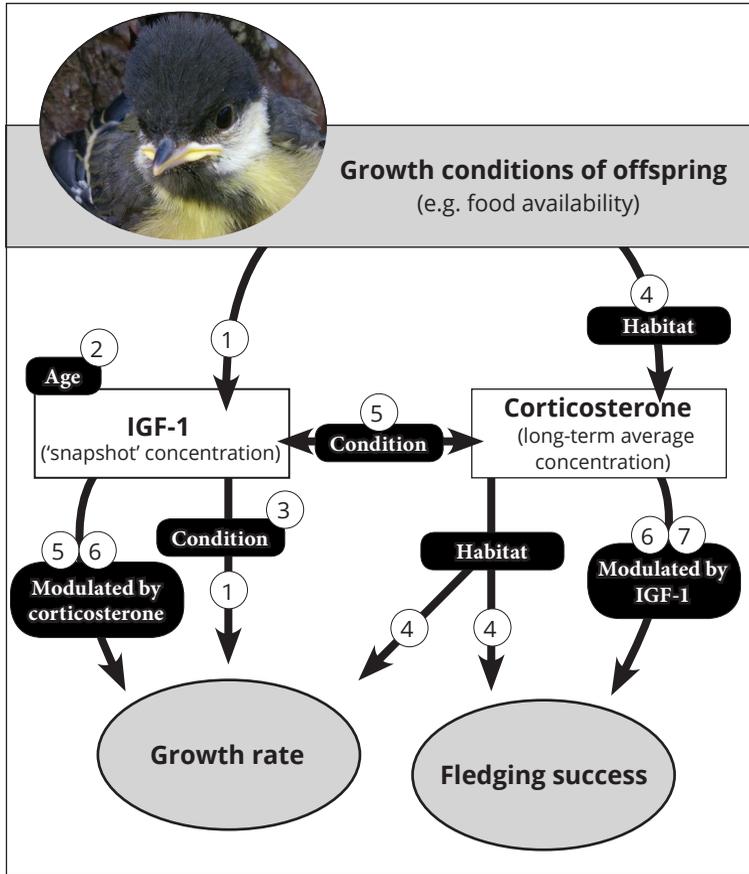
CONCLUSIONS

Figure 8. The main conclusions of the current thesis how insulin-like growth factor I (IGF-1) and corticosterone are associated with nestling growth and fledging success in wild passerines. The black boxes denote the traits that modulate the different associations. The numbers in the hollow circles for every association denote the points that match the numbers in the following conclusion (Photo: Marko Mägi).

- Insulin-like growth factor 1 and fitness of nestlings (see Fig. 8):
  1. Changes in growing conditions (e.g. food availability) for altricial nestlings caused respective changes in their growth rates as could be expected. More interestingly, if nestlings had more food available to them during the fledging period, IGF-1 levels, as an underlying physiological mediator of postnatal growth rate, were also higher than in those nestlings which grew in nutritionally more limited conditions.

2. Plasma IGF-1 levels were higher in the middle of the nestling period (day 7 post-hatch for the pied flycatcher and day 8 post-hatch for the great tit) when growth rate is most rapid, than in the pre-fledging stage (day 13 post-hatch for the pied flycatcher and day 15 post-hatch for the great tit).

3. At the pre-fledging stage, IGF-1 levels were negatively associated with the growth rate of those nestlings that grew in nutritionally better conditions. This indicates an adaptive IGF-1 down-regulation in more mature nestlings that had higher developmental speed and higher IGF-1 levels than smaller-sized less mature nestlings. Therefore, those fledglings that reached optimal fledging mass quicker, probably needed to invest less into somatic growth and structural size at the end of the fledging period. Hence, such nestlings could redirect energy from growth to other functions, thus allowing them to use more resources to cope with the environmental conditions experienced during the transition to independence.

- Corticosterone and fitness of nestlings (see Fig. 8):
  4. Nestlings from enlarged broods had higher corticosterone levels compared to those from control broods and enlarged broods only in coniferous habitat. No differences were found between nestlings from decreased, control and enlarged broods in deciduous habitat. Furthermore, investment into feather corticosterone (potentially enhancing short term survival) is traded off with growth rate, body size at fledging and fledging success of nestlings only in coniferous habitat. Possibly, parents in coniferous forests were not able to provide enough food for enlarged broods, probably due to the limited availability of suitable food items. Overall variability in feather corticosterone levels were also significantly higher in the coniferous habitat in comparison to the deciduous habitat. This suggests that nestlings from such broods may have been chronically stressed, probably owing to dietary restrictions coupled to increased sibling competition. Hence, given that decreased growth rate was associated with lower fledging success, it seems that under poor growth conditions, larger physiological investment into immediate survival might be traded off with longer term survival at the start of independence and later in life.

- Interaction between insulin-like growth factor 1 and corticosterone and fitness of nestlings (see Fig. 8):
  5. It was shown that the signalling pathways of IGF-1 and corticosterone may interact with each other to modulate the early postnatal development in nestlings of a wild passerine. In nestlings that had more food available for them (decreased broods) during the fledging period and grew faster, the association between pre-fledging IGF-1 and feather corticosterone

levels was positive, opposite to that in nestlings that were more nutritionally-restricted during the fledging period (enlarged broods).

6. The results of the thesis suggest that threshold levels of corticosterone, over which its physiological effects switch from stimulatory to inhibitory on the activity of the GH/IGF axis, depend on growth conditions (see Fig. 6). In good conditions, the inflection point of the curve, reflecting the physiological redirection of internal energy sources from development to short term survival, is potentially achieved with relatively higher levels of corticosterone and IGF-1 compared to nestlings in poor conditions.

7. Nutrition-dependent inter-regulation of IGF-1 and corticosterone can be linked to short term fitness, in terms of fledging success, in passerine nestlings.

SUMMARY

Life-history theory seeks answers to questions about how suites of traits, such as growth rate, body mass and survival, have coevolved to maximize the fitness of individuals in their habitat. Postnatal growth rate is an important life-history trait that is strongly associated with survival during the transition to independence and in the overwinter period. In stochastic environments, individual fitness may be closely linked to environmental conditions experienced early in life. When conditions deteriorate, animals have to adapt their physiology accordingly to avoid detrimental effects on growth and survival. Hormones such as insulin-like growth factor 1 (IGF-1) and glucocorticoids are potentially important mediators of developmental plasticity, although their function is quite poorly understood in free-living animals to date.

In this thesis, brood size manipulation and a hormone-injection experiment was conducted in two free-living passerines, the great tit (*Parus major*) and pied flycatcher (*Ficedula hypoleuca*), with the main aim to explore the developmental plasticity of IGF-1 and corticosterone functions in relation to environmental challenges (e.g. changing nutritional conditions).

Results showed that nestlings in better nutritional conditions (decreased broods) had increased postnatal growth rate, better body condition and also higher IGF-1 levels prior to fledging (day 15 post-hatch for the great tit) than those in worse nutritional conditions (control and enlarged broods). It is important to emphasize that IGF-1 is the causal underlying physiological mediator behind the variability in growth rates of passerine nestlings. Plasma IGF-1 levels were higher in the middle of the nestling period (day 7 post-hatch for the pied flycatcher and day 8 post-hatch for the great tit), when growth rate is most rapid, than in the pre-fledging stage (day 13 post-hatch for the pied flycatcher and day 15 post-hatch for the great tit). Interestingly, at the pre-fledging stage, IGF-1 levels were negatively associated with the growth rate of those nestlings that grew in nutritionally better conditions. This indicates an adaptive IGF-1 down-regulation in more mature nestlings that reached optimal fledging mass quicker, and that probably needed to invest less into somatic growth and structural size at the end of the fledging period.

Furthermore, it was shown that feather corticosterone (the measure incorporates an integral hormonal profile over a relatively long time period, during which the feather was growing) and its association with growth rate and fledging success were significantly affected by the treatment only in coniferous forests where growth conditions had a tendency to be poorer than in deciduous forests. More specifically, it was found that feather corticosterone was negatively related to fledging success and unimodally associated with the growth rate in the coniferous habitat. In the latter case, the positive association between growth rate and corticosterone levels became negative as corticosterone levels increased.

Lastly, it was shown that the association between levels of IGF-1 prior to fledging (day 15 post-hatch for the great tit) and feather corticosterone depended on the physiological condition of nestlings. Namely, there was a positive association between the hormone levels in nestlings in better condition from the decreased broods, and a negative association in nestlings in worse condition nestlings from the enlarged broods. Interestingly, the interaction between levels of IGF-1 and corticosterone was also related to the survival of the nestlings.

The results of the current thesis suggest that nestling plasma IGF-1 and integral feather corticosterone levels vary depending on resource (e.g. food) availability. These hormones potentially play an important role in regulating nestling fitness in altricial birds, by mediating physiological trade-offs in the regulation of their postnatal growth rate, physiological condition and fledging success in the stochastic environment.

SUMMARY IN ESTONIAN

Insuliini-laadse kasvufaktori 1 ja kortikosterooni seos värvuliste pesapoegade kasvukiiruse ja lennuvõimestumisedukusega

Elukäiguteooria otsib vastuseid küsimustele, kuidas on erinevad tunnused (nt kasvukiirus, kehakaal ja elumus) koevolutsioneerunud nii, et isendi kohasus tema elukeskkonnas oleks maksimaalne. Sünnijärgne kasvukiirus on oluline elukäigutunnus, mis seostub tugevasti isendi ellujäämusega iseseisva elu alguses ja talvitamisel. Muutlikus keskkonnas sõltub isendi kohasus suure tõenäosusega nendest tingimustest, mis valitsesid tema varase kasvu perioodil. Keskkonnatingimuste halvenedes peavad loomad muutustega füsioloogiliselt kohanema, et vältida negatiivseid mõjusid kasvule ja ellujäämusele. Hormoonid, nagu insuliini-laadne kasvufaktor 1 (IGF-1) ja glükokortikoidid, on olulised arengu plastilisuse füsioloogilised vahendajad, kuid nende täpne funktsioon on vabaltelavail loomadel tänaseni suhteliselt halvasti uuritud.

Töö peamine eesmärk oli testida IGF-1 ja kortikosterooni (lindude peamine glükokortikoid) funktsioonide arengulist plastilisust muutlikes kasvu- ja toitumistingimustes. Selleks viidi läbi pesakonna suuruse manipulatsioon ja IGF-1 süstimise eksperiment vabaltelavatel rasvatihastel (*Parus major*) ja must-kärbsenäppidel (*Ficedula hypoleuca*).

Tulemused näitasid, et paremates toitumistingimustes (vähendatud pesakondade korral) kasvasid uuritud värvuliste pesapojad koorumisjärgselt kiiremini, olid paremas füsioloogilises konditsioonis ja neil oli vahetult enne lennuvõimestumist kõrgem IGF-1 tase, kui halvemates tingimustes (suurendatud pesakondade korral) kasvanud ja kontrollgrupi poegadel. Oluline on siinjuures rõhutada, et IGF-1 on värvuliste kasvukiiruse regulatsiooni kausaalne füsioloogiline vahendaja. Pesapoegade vereplasma IGF-1 tase oli pesaperioodi keskel (7. päeval must-kärbsenäpil; 8. päeval rasvatihasel), mil poegade kasvukiirus on maksimaalne, kõrgem kui lennuvõimestumiseelsel ajal, mil (13. päeval must-kärbsenäpil; 15. päeval rasvatihasel) kasvukiirus on langenud. On märkimisväärne, et lennuvõimestumiseelne IGF-1 tase seostus negatiivselt kasvukiirusega paremates toitumistingimustes kasvanud pesapoegadel. See seos näitab tõenäoliselt adaptiivset IGF-1 allareguleerimist paremini arenenud poegadel, kes saavutasid optimaalse lennuvõimestumiseelse kehamassi kiiremini. Ilmselt ei ole vaja sellistel poegadel investeerida nii palju kasvu ja struktuursesse suurusesse lennuvõimestumisperioodi lõpus kui väiksematel poegadel.

Sulgedes sisalduva kortikosterooni tase (mis tõnäoliselt kajastab hormooni keskmist taset kogu sule kasvamise perioodil) ja selle seos pesapoja kasvukiiruse ning lennuvõimestumisedukusega sõltus pesakonna suuruse manipulatsioonist vaid okasmetsas, kus kasvutingimused kaldusid olema halvemad kui lehtmetsas. Täpsemalt seostus sulgedes sisalduva kortikosterooni tase okasmetsas negatiivselt lennuvõimestumisedukusega ja paraboolse kõvera kohaselt kasvukiirusega. Viimasel juhul oli kortikosterooni madala taseme korral kasvu-

kiirus sellega seotud positiivselt, kuid hormooni taseme suurenedes muutus seos negatiivseks.

Seos poegade lennuvõimestumiseelse (15. päev rasvatihasel) IGF-1 ja sule kortikosterooni tasemete vahel sõltus pesapoegade füsioloogilisest konditsioonist. Nimelt oli nende kahe hormooni tasemete vahel positiivne seos heas konditsioonis pesapoegadel vähendatud suurusega pesakondades ja negatiivne seos halvas konditsioonis pesapoegadel suurendatud pesakondades. Väärib märkimist, et IGF-1 ja kortikosterooni tasemete koosmõju oli seotud ka poegade lennuvõimestumisedukusega.

Antud töö tulemused näitavad, et värvuliste pesapoegade vereplasma IGF-1 ja sulgedes sisalduva kortikosterooni tasemed varieeruvad vastavalt kasvutingimustele (nt toidu kättesaadavusele). Tõenäoliselt mängivad uuritud hormoonid tähtsat rolli pesahoidjate lindude poegade kohasuse varieeruvuses, seda läbi füsioloogiliste lõivsuhete nende sünnijärgse kasvukiiruse, füsioloogilise konditsiooni ja lennuvõimestumisedukuse regulatsioonis muutlikes keskkonnatingimustes.

REFERENCES

Anacker, C., Cattaneo, A., Luoni, A., Musaelyan, K., Zunszain, P.A., Milanesi, E., Rybka, J., Berry, A., Cirulli, F. & Thuret, S. (2013) Glucocorticoid-related molecular signaling pathways regulating hippocampal neurogenesis. *Neuropsychopharmacology,* **38,** 872–883.

Angelier, F., Wingfield, J.C., Weimerskirch, H. & Chastel, O. (2010) Hormonal correlates of individual quality in a long-lived bird: a test of the 'corticosterone–fitness hypothesis'. *Biology Letters,* **6,** 846–849.

Bassas, L., Lesniak, M.A., Serrano, J., Roth, J. & De Pablo, F. (1988) Developmental Regulation of Insulin and Type I Insulin-Like Growth Factor Receptors and Absence of Type II Receptors in Chicken Embryo Tissues. *Diabetes,* **37,** 637–644.

Beccavin, C., Chevalier, B., Cogburn, L., Simon, J. & Duclos, M. (2001) Insulin-like growth factors and body growth in chickens divergently selected for high or low growth rate. *Journal of Endocrinology,* **168,** 297–306.

Beckman, B.R. (2011) Perspectives on concordant and discordant relations between insulin-like growth factor 1 (IGF1) and growth in fishes. *General and Comparative Endocrinology,* **170,** 233–252.

Belanto, J.J., Diaz-Perez, S.V., Magyar, C.E., Maxwell, M.M., Yilmaz, Y., Topp, K., Boso, G., Jamieson, C.H., Cacalano, N.A. & Jamieson, C.A.M. (2010) Dexamethasone induces dysferlin in myoblasts and enhances their myogenic differentiation. *Neuromuscular Disorders,* **20,** 111–121.

Bellows, C.G., Aubin, J.E. & Heersche, J.N.M. (1987) Physiological Concentrations of Glucocorticoids Stimulate Formation of Bone Nodules from Isolated Rat Calvaria Cells in Vitro. *Endocrinology,* **121,** 1985–1992.

Blas, J., Bortolotti, G.R., Tella, J.L., Baos, R. & Marchant, T.A. (2007) Stress response during development predicts fitness in a wild, long lived vertebrate. *Proceedings of the National Academy of Sciences of the United States of America,* **104,** 8880–8884.

Bondy, C.A. & Cheng, C.M. (2004) Signaling by insulin-like growth factor 1 in brain. *European Journal of Pharmacology,* **490,** 25–31.

Bonier, F., Martin, P.R., Moore, I.T. & Wingfield, J.C. (2009) Do baseline glucocorticoids predict fitness? *Trends in Ecology & Evolution,* **24,** 634–642.

Boonstra, R. (2013) Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. *Functional Ecology,* **27,** 11–23.

Bortolotti, G.R., Marchant, T., Blas, J. & Cabezas, S. (2009) Tracking stress: localisation, deposition and stability of corticosterone in feathers. *Journal of Experimental Biology,* **212,** 1477–1482.

Bortolotti, G.R., Marchant, T.A., Blas, J. & German, T. (2008) Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. *Functional Ecology,* **22,** 494–500.

Bossis, I. & Porter, T.E. (2003) Evaluation of glucocorticoid-induced growth hormone gene expression in chicken embryonic pituitary cells using a novel in situ mRNA quantitation method. *Molecular and Cellular Endocrinology,* **201,** 13–23.

Breier, B.H. (1999) Regulation of protein and energy metabolism by the somatotropic axis. *Domestic Animal Endocrinology,* **17,** 209–218.

Buyse, J. & Decuypere, E. (1999) The role of the somatotrophic axis in the metabolism of the chicken. *Domestic Animal Endocrinology,* **17,** 245–255.

Canalis, E., Mazziotti, G., Giustina, A. & Bilezikian, J.P. (2007) Glucocorticoid-induced osteoporosis: pathophysiology and therapy. *Osteoporosis International,* **18,** 1319–1328.

Capellan, E. & Nicieza, A. (2007) Non-equivalence of growth arrest induced by predation risk or food limitation: context-dependent compensatory growth in anuran tadpoles. *Journal of Animal Ecology,* **76,** 1026–1035.

Cassy, S., Derouet, M., Crochet, S., Dridi, S. & Taouis, M. (2003) Leptin and insulin downregulate leptin receptor gene expression in chicken-derived leghorn male hepatoma cells. *Poultry Science,* **82,** 1573–1579.

Castellano, G., Affuso, F., Di Conza, P. & Fazio, S. (2009) The GH/IGF-1 Axis and Heart Failure. *Current Cardiology Reviews,* **5,** 203–215.

Cianfarani, S., Germani, D., Rossi, L., Argiro, G., Boemi, S., Lemon, M., Holly, J. & Branca, F. (1998) IGF-I and IGF-binding protein-1 are related to cortisol in human cord blood. *European Journal of Endocrinology,* **138,** 524–529.

Dantzer, B. & Swanson, E.M. (2012) Mediation of vertebrate life histories via insulin-like growth factor-1. *Biological Reviews,* **87,** 414–429.

Daza, D.O., Sundstrom, G., Bergqvist, C.A., Duan, C. & Larhammar, D. (2011) Evolution of the Insulin-Like Growth Factor Binding Protein (IGFBP) Family. *Endocrinology,* **152,** 2278–2289.

Dufty Jr, A.M., Clobert, J. & Møller, A.P. (2002) Hormones, developmental plasticity and adaptation. *Trends in Ecology & Evolution,* **17,** 190–196.

Fairhurst, G.D., Marchant, T.A., Soos, C., Machin, K.L. & Clark, R.G. (2013) Experimental relationships between levels of corticosterone in plasma and feathers in a free-living bird. *Journal of Experimental Biology,* **216,** 4071–4081.

Fingar, D.C. & Blenis, J. (2004) Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. *Oncogene,* **23,** 3151–3171.

Flatt, T. & Heyland, A. (2011) *Mechanisms of Life History Evolution: The Genetics and Physiology of Life History Traits and Trade-Offs*. Oxford University Press, Oxford.

Giachetto, P.F., Riedel, E.C., Gabriel, J.E., Ferro, M.I.T., Di Mauro, S.M.Z., Macari, M. & Ferro, J.A. (2004) Hepatic mRNA expression and plasma levels of insulin-like growth factor-I (IGF-I) in broiler chickens selected for different growth rates. *Genetics and Molecular Biology,* **27,** 39–44.

Goutte, A., Angelier, F., Welcker, J., Moe, B., Clément-Chastel, C., Gabrielsen, G.W., Bech, C. & Chastel, O. (2010) Long-term survival effect of corticosterone manipulation in Black-legged kittiwakes. *General and Comparative Endocrinology,* **167,** 246–251.

Heath, J. (1997) Corticosterone levels during nest departure of juvenile American kestrels. *Condor,* **99,** 806–811.

Heath, J.A. & Dufty, A.M. (1998) Body Condition and the Adrenal Stress Response in Captive American Kestrel Juveniles. *Physiological Zoology,* **71,** 67–73.

Ho, K.Y., Veldhuis, J.D., Johnson, M.L., Furlanetto, R., Evans, W.S., Alberti, K.G.M.M. & Thorner, M.O. (1988) Fasting enhances growth hormone secretion and amplifies the complex rhythms of growth hormone secretion in man. *Journal of Clinical Investigation,* **81,** 968–975.

Howard, E. & Benjamins, J.A. (1975) DNA, ganglioside and sulfatide in brains of rats given corticosterone in infancy, with an estimate of cell loss during development. *Brain Research,* **92,** 73–87.

Huybrechts, L.M., Decuypere, E., Buyse, J., Kühn, E.R. & Tixier-boichard, M. (1992) Effect of Recombinant Human Insulin-Like Growth Factor-I on Weight Gain, Fat Content, and Hormonal Parameters in Broiler Chickens. *Poultry Science,* **71,** 181–187.

Hwa, V., Oh, Y. & Rosenfeld, R.G. (1999) The insulin-like growth factor-binding protein (IGFBP) superfamily. *Endocrine Reviews,* **20,** 761–787.

Hõrak, P., Tegelmann, L., Ots, I. & Møller, A.P. (1999) Immune function and survival of great tit nestlings in relation to growth conditions. *Oecologia,* **121,** 316–322.

Inui, A. (2001) Ghrelin: An orexigenic and somatotrophic signal from the stomach. *Nature Reviews: Neuroscience,* **2,** 551–560.

Jux, C., Leiber, K., Hügel, U., Blum, W., Ohlsson, C., Klaus, G. & Mehls, O. (1998) Dexamethasone Impairs Growth Hormone (GH)-Stimulated Growth by Suppression of Local Insulin-Like Growth Factor (IGF)-I Production and Expression of GH- and IGF-I-Receptor in Cultured Rat Chondrocytes. *Endocrinology,* **139,** 3296–3305.

Kaiya, H., Kangawa, K. & Miyazato, M. (2013) What is the general action of ghrelin for vertebrates? – Comparisons of ghrelin's effects across vertebrates. *General and Comparative Endocrinology,* **181,** 187–191.

Kajimura, S., Hirano, T., Visitacion, N., Moriyama, S., Aida, K. & Grau, E. (2003) Dual mode of cortisol action on GH/IGF-I/IGF binding proteins in the tilapia, Oreochromis mossambicus. *Journal of Endocrinology,* **178,** 91–99.

Kern, M., Bacon, W., Long, D. & Cowie, R.J. (2001) Possible roles for corticosterone and critical size in the fledging of nestling pied flycatchers. *Physiological and Biochemical Zoology,* **74,** 651–659.

Kitaysky, A.S., Kitaiskaia, E.V., Piatt, J.F. & Wingfield, J.C. (2003) Benefits and costs of increased levels of corticosterone in seabird chicks. *Hormones and Behavior,* **43,** 140–149.

Kitaysky, A.S., Wingfield, J.C. & Piatt, J.F. (2001) Corticosterone facilitates begging and affects resource allocation in the black-legged kittiwake. *Behavioral Ecology,* **12,** 619–625.

Klaus, G., Jux, C., Fernandez, P., Rodriguez, J., Himmele, R. & Mehls, O. (2000) Suppression of growth plate chondrocyte proliferation by corticosteroids. *Pediatric Nephrology,* **14,** 612–615.

Kocamis, H., Kirkpatrick-Keller, D., Klandorf, H. & Killefer, J. (1998) In ovo administration of recombinant human insulin-like growth factor-I alters postnatal growth and development of the broiler chicken. *Poultry Science,* **77,** 1913–1919.

Kuo, T., McQueen, A., Chen, T.-C. & Wang, J.-C. (2015) Regulation of glucose homeostasis by glucocorticoids. *Glucocorticoid Signaling*, pp. 99–126. Springer.

Landys, M.M., Ramenofsky, M. & Wingfield, J.C. (2006) Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *General and Comparative Endocrinology,* **148,** 132–149.

Latres, E., Amini, A.R., Amini, A.A., Griffiths, J., Martin, F.J., Wei, Y., Lin, H.C., Yancopoulos, G.D. & Glass, D.J. (2005) Insulin-like Growth Factor-1 (IGF-1) Inversely Regulates Atrophy-induced Genes via the Phosphatidylinositol 3-Kinase/ Akt/Mammalian Target of Rapamycin (PI3K/Akt/mTOR) Pathway. *Journal of Biological Chemistry,* **280,** 2737–2744.

Lattin, C.R., Reed, J.M., DesRochers, D.W. & Romero, L.M. (2011) Elevated corticosterone in feathers correlates with corticosterone-induced decreased feather quality: a validation study. *Journal of Avian Biology,* **42,** 247–252.

Li, R., Hu, Y., Ni, Y., Xia, D., Grossmann, R. & Zhao, R. (2011) Leptin stimulates hepatic activation of thyroid hormones and promotes early posthatch growth in the chicken. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* **160,** 200–206.

Liu, H.H., Wang, J.W., Chen, X., Zhang, R.P., Yu, H.Y., Jin, H.B., Li, L. & Han, C.C. (2011) In ovo administration of rhIGF-1 to duck eggs affects the expression of myogenic transcription factors and muscle mass during late embryo development. *Journal of Applied Physiology,* **111,** 1789–1797.

Liu, J.L. & LeRoith, D. (1999) Insulin-like growth factor I is essential for postnatal growth in response to growth hormone. *Endocrinology,* **140,** 5178–5184.

Liu, J.L., Yakar, S. & Leroith, D. (2000) Conditional knockout of mouse insulin-like growth factor-1 gene using the Cre/loxP system. *Proceedings of the Society for Experimental Biology and Medicine,* **223,** 344–351.

Liu, J.P., Baker, J., Perkins, A.S., Robertson, E.J. & Efstratiadis, A. (1993) Mice carrying null mutations of the genes encoding insulin-like growth factor-i (igf-1) and type-1 igf receptor (igf1r). *Cell,* **75,** 59–72.

Loiseau, C., Sorci, G., Dano, S. & Chastel, O. (2008) Effects of experimental increase of corticosterone levels on begging behavior, immunity and parental provisioning rate in house sparrows. *General and Comparative Endocrinology,* **155,** 101–108.

Love, O.P., McGowan, P.O. & Sheriff, M.J. (2013) Maternal adversity and ecological stressors in natural populations: the role of stress axis programming in individuals, with implications for populations and communities. *Functional Ecology,* **27,** 81–92.

Lupu, F., Terwilliger, J.D., Lee, K., Segre, G.V. & Efstratiadis, A. (2001) Roles of Growth Hormone and Insulin-like Growth Factor 1 in Mouse Postnatal Growth. *Developmental Biology,* **229,** 141–162.

Maness, T.J. & Anderson, D.J. (2013) Predictors of Juvenile Survival in Birds. *Ornithological Monographs,* **78,** 1–55.

Martial, J.A., Seeburg, P.H., Guenzi, D., Goodman, H.M. & Baxter, J.D. (1977) Regulation of growth hormone gene expression: synergistic effects of thyroid and glucocorticoid hormones. *Proceedings of the National Academy of Sciences of the United States of America,* **74,** 4293–4295.

Martin, L.B. (2009) Stress and immunity in wild vertebrates: Timing is everything. *General and Comparative Endocrinology,* **163,** 70–76.

Martin, T.E. (1987) Food as a limit on breeding birds: a life-history perspective. *Annual Review of Ecology and Systematics,* **18,** 453–487.

Mazziotti, G. & Giustina, A. (2013) Glucocorticoids and the regulation of growth hormone secretion. *Nature Reviews: Endocrinology,* **9,** 265–276.

McCarthy, T.L., Centrella, M. & Canalis, E. (1990) Cortisol Inhibits the Synthesis of Insulin-Like Growth Factor-I in Skeletal Cells. *Endocrinology,* **126,** 1569–1575.

McEwen, B.S., Biron, C.A., Brunson, K.W., Bulloch, K., Chambers, W.H., Dhabhar, F.S., Goldfarb, R.H., Kitson, R.P., Miller, A.H., Spencer, R.L. & Weiss, J.M. (1997) The role of adrenocorticoids as modulators of immune function in health and disease: Neural, endocrine and immune interactions. *Brain Research Reviews,* **23,** 79–133.

McEwen, B.S. & Wingfield, J.C. (2003) The concept of allostasis in biology and bio-medicine. *Hormones and Behavior,* **43,** 2–15.

Mehls, O., Irzynjec, T., Ritz, E., Eden, S., Kovacs, G., Klaus, G., Floege, J. & Mall, G. (1993) Effects of rhGH and rhIGF-1 on renal growth and morphology. *Kidney International,* **44,** 1251–1258.

Mitchell, G.W., Guglielmo, C.G., Wheelwright, N.T., Freeman-Gallant, C.R. & Norris, D.R. (2011) Early Life Events Carry Over to Influence Pre-Migratory Condition in a Free-Living Songbird. *PloS One,* **6,** e28838.

Mohan, S. & Kesavan, C. (2012) Role of Insulin-like Growth Factor-1 in the Regulation of Skeletal Growth. *Current Osteoporosis Reports,* **10,** 178–186.

Morgan, S.A., Gathercole, L.L., Simonet, C., Hassan-Smith, Z.K., Bujalska, I., Guest, P., Abrahams, L., Smith, D.M., Stewart, P.M., Lavery, G.G. & Tomlinson, J.W. (2013) Regulation of Lipid Metabolism by Glucocorticoids and 11β-HSD1 in Skeletal Muscle. *Endocrinology,* **154,** 2374–2384.

Morici, L.A., Elsey, R.M. & Lance, V.A. (1997) Effects of long-term corticosterone implants on growth and immune function in juvenile alligators, Alligator mississippiensis. *Journal of Experimental Zoology,* **279,** 156–162.

Mägi, M. & Mänd, R. (2004) Habitat differences in allocation of eggs between successive breeding attempts in great tits (*Parus major*). *Ecoscience,* **11,** 361–369.

Mägi, M., Mänd, R., Tamm, H., Sisask, E., Kilgas, P. & Tilgar, V. (2009) Low reproductive success of great tits in the preferred habitat: A role of food availability. *Ecoscience,* **16,** 145–157.

Mänd, R., Tilgar, V., Lohmus, A. & Leivits, A. (2005) Providing nest boxes for hole-nesting birds – Does habitat matter? *Biodiversity and Conservation,* **14,** 1823–1840.

Naef-Daenzer, B., Widmer, F. & Nuber, M. (2001) Differential post-fledging survival of great and coal tits in relation to their condition and fledging date. *Journal of Animal Ecology,* **70,** 730–738.

Nakae, J., Kido, Y. & Accili, D. (2001) Distinct and Overlapping Functions of Insulin and IGF-I Receptors. *Endocrine Reviews,* **22,** 818–835.

Nanto-Salonen, K., Muller, H.L., Hoffman, A.R., Vu, T.H. & Rosenfeld, R.G. (1993) Mechanisms of thyroid hormone action on the insulin-like growth factor system: All thyroid hormone effects are not growth hormone mediated. *Endocrinology,* **132,** 781–788.

Neuenschwander, S., Brinkhof, M.W.G., Kölliker, M. & Richner, H. (2003) Brood size, sibling competition, and the cost of begging in great tits (*Parus major*). *Behavioral Ecology,* **14,** 457–462.

Newton, I. (1998) *Population Limitation in Birds*. Academic Press, San Diego, CA.

Nilsson, J.-Å. & Gårdmark, A. (2001) Sibling competition affects individual growth strategies in marsh tit, *Parus palustris*, nestlings. *Animal Behaviour,* **61,** 357–365.

O'Kusky, J.R., Ye, P. & D'Ercole, A.J. (2000) Insulin-like growth factor-I promotes neurogenesis and synaptogenesis in the hippocampal dentate gyrus during postnatal development. *Journal of Neuroscience,* **20,** 8435–8442.

Otto, A. & Patel, K. (2010) Signalling and the control of skeletal muscle size. *Experimental Cell Research,* **316,** 3059–3066.

Pansters, N.A., Langen, R.C., Wouters, E.F. & Schols, A.M. (2013) Synergistic stimulation of myogenesis by glucocorticoid and IGF-I signaling. *Journal of Applied Physiology,* **114,** 1329–1339.

Patterson, A.G.L., Kitaysky, A.S., Lyons, D.E. & Roby, D.D. (2015) Nutritional stress affects corticosterone deposition in feathers of Caspian tern chicks. *Journal of Avian Biology,* **46,** 18–24.

Paulsen, S.K., Pedersen, S.B., Jørgensen, J.O.L., Fisker, S., Christiansen, J.S., Flyvbjerg, A. & Richelsen, B. (2006) Growth Hormone (GH) Substitution in GH-Deficient Patients Inhibits 11β-Hydroxysteroid Dehydrogenase Type 1 Messenger

Ribonucleic Acid Expression in Adipose Tissue. *The Journal of Clinical Endocrinology & Metabolism,* **91,** 1093–1098.

Pettifor, R.A., Perrins, C.M. & McCleery, R.H. (2001) The individual optimization of fitness: variation in reproductive output, including clutch size, mean nestling mass and offspring recruitment, in manipulated broods of great tits *Parus major*. *Journal of Animal Ecology,* **70,** 62–79.

Pickering, A. (1984) Cortisol-induced lymphocytopenia in brown trout, Salmo trutta L. *General and Comparative Endocrinology,* **53,** 252–259.

Rahimi, G. (2005) Characterization of GH, GHR and IGF-I in Broiler Lines Selected for Rapid Growth or Improved Feed Efficiency. *International Journal of Poultry Science,* **4,** 476–481.

Rajaram, S., Baylink, D.J. & Mohan, S. (1997) Insulin-Like Growth Factor-Binding Proteins in Serum and Other Biological Fluids: Regulation and Functions. *Endocrine Reviews,* **18,** 801–831.

Ralphs, J.R., Wylie, L. & Hill, D.J. (1990) Distribution of insulin-like growth factor peptides in the developing chick embryo. *Development,* **109,** 51–58.

Remmel, T., Tammaru, T. & Mägi, M. (2009) Seasonal mortality trends in tree-feeding insects: A field experiment. *Ecological Entomology,* **34,** 98–106.

Ren, J., Samson, W.K. & Sowers, J.R. (1999) Insulin-like Growth Factor I as a Cardiac Hormone: Physiological and Pathophysiological Implications in Heart Disease. *Journal of Molecular and Cellular Cardiology,* **31,** 2049–2061.

Rivers, J.W., Liebl, A.L., Owen, J.C., Martin, L.B. & Betts, M.G. (2012) Baseline corticosterone is positively related to juvenile survival in a migrant passerine bird. *Functional Ecology,* **26,** 1127–1134.

Robson, H., Siebler, T., Shalet, S.M. & Williams, G.R. (2002) Interactions between GH, IGF-I, glucocorticoids, and thyroid hormones during skeletal growth. *Pediatric Research,* **52,** 137–147.

Romero, L.M. (2004) Physiological stress in ecology: Lessons from biomedical research. *Trends in Ecology & Evolution,* **19,** 249–255.

Ross, R.J. & Buchanan, C.R. (1990) Growth hormone secretion: its regulation and the influence of nutritional factors. *Nutrition research reviews,* **3,** 143–162.

Saaltink, D.-J. & Vreugdenhil, E. (2014) Stress, glucocorticoid receptors, and adult neurogenesis: a balance between excitation and inhibition? *Cellular and Molecular Life Sciences,* **71,** 2499–2515.

Sanz, J.J. & Tinbergen, J.M. (1999) Energy expenditure, nestling age, and brood size: an experimental study of parental behavior in the great tit *Parus major*. *Behavioral Ecology,* **10,** 598–606.

Sapolsky, R.M., Romero, L.M. & Munck, A.U. (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews,* **21,** 55–89.

Scacchi, M., Pincelli, A.I. & Cavagnini, F. (2003) Nutritional status in the neuroendocrine control of growth hormone secretion: The model of anorexia nervosa. *Frontiers in Neuroendocrinology,* **24,** 200–224.

Schlueter, P.J., Peng, G., Westerfield, M. & Duan, C. (2007) Insulin-like growth factor signaling regulates zebrafish embryonic growth and development by promoting cell survival and cell cycle progression. *Cell Death and Differentiation,* **14,** 1095–1105.

Schulkin, J. (2011) Evolutionary conservation of glucocorticoids and corticotropin releasing hormone: behavioral and physiological adaptations. *Brain Research,* **1392,** 27–46.

Schultner, J., Kitaysky, A.S., Welcker, J. & Hatch, S. (2013) Fat or lean: adjustment of endogenous energy stores to predictable and unpredictable changes in allostatic load. *Functional Ecology,* **27,** 45–55.

Siegel, H.S. (1980) Physiological Stress in Birds. *Bioscience,* **30,** 529–534.

Sisask, E., Mänd, R., Mägi, M. & Tilgar, V. (2010) Parental provisioning behaviour in Pied Flycatchers *Ficedula hypoleuca* is well adjusted to local conditions in a mosaic of deciduous and coniferous habitat. *Bird Study,* **57,** 447–457.

Sjögren, K., Wallenius, K., Liu, J.-L., Bohlooly-Y, M., Pacini, G., Svensson, L., Törnell, J., Isaksson, O.G.P., Ahrén, B., Jansson, J.-O. & Ohlsson, C. (2001) Liver-Derived IGF-I is of Importance for Normal Carbohydrate and Lipid Metabolism. *Diabetes,* **50,** 1539–1545.

Smith, R. (1990) Corticosteroids and osteoporosis. *Thorax,* **45,** 573–578.

Stearns, S.C. (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford.

Stitt, T.N., Drujan, D., Clarke, B.A., Panaro, F., Timofeyva, Y., Kline, W.O., Gonzalez, M., Yancopoulos, G.D. & Glass, D.J. (2004) The IGF-1/PI3K/Akt Pathway Prevents Expression of Muscle Atrophy-Induced Ubiquitin Ligases by Inhibiting FOXO Transcription Factors. *Molecular Cell,* **14,** 395–403.

Stratikopoulos, E., Szabolcs, M., Dragatsis, I., Klinakis, A. & Efstratiadis, A. (2008) The hormonal action of IGF1 in postnatal mouse growth. *Proceedings of the National Academy of Sciences,* **105,** 19378–19383.

Sui, N., Sandi, C. & Rose, S.P. (1997) Interactions of corticosterone and embryonic light deprivation on memory retention in day-old chicks. *Developmental Brain Research,* **101,** 269–272.

Zanette, L., Clinchy, M. & Smith, J.N. (2006) Combined food and predator effects on songbird nest survival and annual reproductive success: results from a bi-factorial experiment. *Oecologia,* **147,** 632–640.

Zera, A.J. & Harshman, L.G. (2001) The physiology of life history trade-offs in animals. *Annual Review of Ecology and Systematics,* **32,** 95–126.

Zheng, J., Takagi, H., Tsutsui, C., Adachi, A. & Sakai, T. (2008) Hypophyseal corticosteroids stimulate somatotrope differentiation in the embryonic chicken pituitary gland. *Histochemistry and Cell Biology,* **129,** 357–365.

Takaya, K., Ariyasu, H., Kanamoto, N., Iwakura, H., Yoshimoto, A., Harada, M., Mori, K., Komatsu, Y., Usui, T., Shimatsu, A., Ogawa, Y., Hosoda, K., Akamizu, T., Kojima, M., Kangawa, K. & Nakao, K. (2000) Ghrelin Strongly Stimulates Growth Hormone Release in Humans. *The Journal of Clinical Endocrinology & Metabolism,* **85,** 4908–4911.

Tarwater, C.E., Ricklefs, R.E., Maddox, J.D. & Brawn, J.D. (2010) Pre-reproductive survival in a tropical bird and its implications for avian life histories. *Ecology,* **92,** 1271–1281.

Tilgar, V., Kilgas, P., Mägi, M. & Mänd, R. (2008) Age-related changes in the activity of bone alkaline phosphatase and its application as a marker of prefledging maturity of nestlings in wild passerines. *Auk,* **125,** 456–460.

Tilgar, V. & Mänd, R. (2006) Sibling growth patterns in great tits: Does increased selection on last-hatched chicks favour an asynchronous hatching strategy? *Evolutionary Ecology,* **20,** 217–234.

Tilgar, V., Mänd, R. & Mägi, M. (2002) Calcium shortage as a constraint on reproduction in great tits *Parus major*: A field experiment. *Journal of Avian Biology,* **33,** 407–413.

Tinbergen, J.M. (1987) Costs of reproduction in the great tit − intraseasonal costs associated with brood size. *Ardea,* **75,** 111–122.

Tixier-Boichard, M., Huybrechts, L.M., Decuypere, E., Kühn, E.R., Monvoisin, J.-L., Coquerelle, G., Charrier, J. & Simon, J. (1992) Effects of insulin-like growth factor-I (IGF-I) infusion and dietary tri-iodothyronine (T3) supplementation on growth, body composition and plasma hormone levels in sex-linked dwarf mutant and normal chickens. *Journal of Endocrinology,* **133,** 101–110.

Unterman, T.G., Jentel, J.J., Oehler, D.T., Lacson, R.G. & Hofert, J.F. (1993) Effects of glucocorticoids on circulating levels and hepatic expression of insulin-like growth factor (IGF)-binding proteins and IGF-I in the adrenalectomized streptozotocin-diabetic rat. *Endocrinology,* **133,** 2531–2539.

Wada, H. & Breuner, C.W. (2008) Transient elevation of corticosterone alters begging behavior and growth of white-crowned sparrow nestlings. *Journal of Experimental Biology,* **211,** 1696–1703.

van Balen, J.H. (1973) A Comparative Sudy of the Breeding Ecology of the Great Tit *Parus major* in Different Habitats. *Ardea,* **61,** 1–93.

Wang, G.-s., Liu, H.-h., Li, L.-s. & Wang, J.-w. (2012) Influence of Ovo Injecting IGF-1 on Weights of Embryo, Heart and Liver of Duck During Hatching Stages. *International Journal of Poultry Science,* **11,** 756–760.

Will, A.P., Suzuki, Y., Elliott, K.H., Hatch, S.A., Watanuki, Y. & Kitaysky, A.S. (2014) Feather corticosterone reveals developmental stress in seabirds. *Journal of Experimental Biology,* **217,** 2371–2376.

Wren, A.M., Seal, L.J., Cohen, M.A., Brynes, A.E., Frost, G.S., Murphy, K.G., Dhillo, W.S., Ghatei, M.A. & Bloom, S.R. (2001) Ghrelin Enhances Appetite and Increases Food Intake in Humans. *The Journal of Clinical Endocrinology & Metabolism,* **86,** 5992–5992.

Xu, X., Capito, R.M. & Spector, M. (2008) Delivery of plasmid IGF-1 to chondrocytes via cationized gelatin nanoparticles. *Journal of Biomedical Materials Research Part A,* **84A,** 73–83.

Yakar, S., Rosen, C.J., Beamer, W.G., Ackert-Bicknell, C.L., Wu, Y., Liu, J.L., Ooi, G.T., Setser, J., Frystyk, J., Boisclair, Y.R. & LeRoith, D. (2002) Circulating levels of IGF-1 directly regulate bone growth and density. *Journal of Clinical Investigation,* **110,** 771–781.

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Conference presentations:
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4. Lodjak, J. & Mägi, M. Insulin-like growth factor 1, corticosterone and growth rate of nestlings in wild passerines. Käitumise ja Füsioloogilise Ökoloogia Sümpoosium (8. detsember, 2015). Groningen, Holland. Suuline ettekanne.

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