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Food restriction in young Japanese quails: effects on growth, metabolism, plasma thyroid hormones and mRNA species in the thyroid hormone signalling pathway

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SUMMARY

Young birds, in their post-natal growth period, may reduce their growth and metabolism when facing a food shortage. To examine how such responses can be mediated by endocrine-related factors, we exposed Japanese quail chicks to food restriction for either 2 days (age 6–8 days) or 5 days (age 6–11 days). We then measured growth and resting metabolic rate (RMR), and circulating 3,3′,5-triiodo-L-thyronine (T3) and 3,5,3′,5′-tetraiodothyronine (T4) levels as well as expression patterns of genes involved in growth (insulin-like growth factor-I: IGF-I) and thyroid hormone signalling (thyroid-stimulating hormone-β: TSHβ, type II iodothyronine deiodinase: D2, thyroid hormone receptors isoforms: TRα and TRβ). The food-restricted chicks receiving a weight-maintenance diet showed reductions in structural growth and RMR. Plasma levels of both T3 and T4 were reduced in the food-restricted birds, and within the 5 days food-restricted group there was a positive correlation between RMR and T3. IGF-I mRNA showed significantly higher abundance in the liver of *ad libitum* fed birds at day 8 compared with food-restricted birds. In the brain, TSHβ mRNA level tended to be lower in food-restricted quails on day 8 compared with controls. Furthermore, TRα expression was lower in the brain of food-restricted birds at day 8 compared with birds fed *ad libitum*. Interestingly, brain D2 mRNA was negatively correlated with plasma T3 levels, tending to increase with the length of food restriction. Overall, our results show that food restriction produced significant effects on circulating thyroid hormones and differentially affected mRNA species in the thyroid hormone signalling pathway. Thus, we conclude that the effects of food restriction observed on growth and metabolism were partly mediated by changes in the endocrine-related factors investigated.

INTRODUCTION

The transition from neonate to adult involves both growth and maturation and is consequently a crucial stage in the life of any organism, including birds. Birds are inevitably influenced by their environment and may be especially vulnerable to fluctuating environmental conditions early in life during the period of development. Encountering unfavourable environmental conditions may cause the phenotype to deviate from normal ontogenetic development. Such early life environmentally induced phenotypic deviation is known as developmental plasticity (Smith-Gill, 1983; West-Eberhard, 2003).

A period of low food availability is a challenge that young birds are likely to experience (Schew and Ricklefs, 1998). During development, chicks need to allocate their available energy between maintenance, growth and maturation, and food availability consequently plays a crucial role during this period (Martin, 1987). Depending on the amount of stored reserves and how energy is allocated between different energy-consuming tasks, growth and maturation will sooner or later decline in chicks facing severe food shortage. In addition, chicks have also been shown to lower their maintenance cost, i.e. resting metabolic rate (RMR), during periods of temporal food shortage (Brzek and Konarzewski, 2001; Moe et al., 2004; Moe et al., 2005a; Moe et al., 2005b; Schew, 1995).

Phenotypic deviation in growth and metabolism elicited by unfavourable environmental conditions is usually a product of changes in physiological signals acting downstream on these parameters. For example, the profound effects of poor feeding condition on metabolism and growth are likely to be mediated by the endocrine system. Thyroid hormones, growth hormone and insulin-like growth factors (IGFs) are all known to be key hormones influencing growth and maturation as well as metabolism, and the ontogeny of these hormones and their downstream effects has been thoroughly investigated, especially in precocial bird species (McNabb et al., 1998; McNabb, 2006). Food restriction has repeatedly been shown to influence plasma concentration of these hormones (e.g. Schew et al., 1996; Van der Geyten et al., 1999). However, the relationship between nutritional status and developmental plasticity cannot be fully understood by studying changes in plasma hormone concentrations alone. Hormones may interact in multiple pathways and the observed effects depend on the availability of several other endogenous substances such as hormone binding proteins, hormone receptors and metabolic enzymes (Decuypere et al., 2005). Recent studies on precocial bird species showed that food restriction produced effects on expression levels of genes in these hormone systems. For example, the expression of type III iodothyronine deiodinase, which inactivates thyroid hormones, increases in the liver (van der Geyten et al., 1999) as a response to food restriction. Whereas the mRNA levels of IGF-I, which is important for normal growth and development, decrease in the liver as a response to food restriction (Beccavin et al., 2001;

Kita et al., 2005). Additionally, food restriction has been observed to potentially influence thyroid hormone release through reduction in thyroid-stimulating hormone β (TSHβ) mRNA expression in the pituitary gland (Kobayashi and Ishii, 2002). Thus, nutritional effects on growth and metabolism appear to be partly regulated through changes in mRNA expression of important endocrine factors. Hence, understanding the interaction between changes at different levels of physiological parameters is a prerequisite to understanding the mechanisms underlying phenotypic changes triggered by undernourishment.

In the present study, our aim was to examine the effect of food restriction on growth and metabolism in relation to changes in lower physiological traits believed to influence growth and metabolic development. In order to realise these objectives, we have measured RMR, body mass and organ mass (liver and kidney), and plasma 3′3′5-triiodo-L-thyronine (T3) and 3′5′3′5′-tetraiodothyronine (T4) levels in *ad libitum* fed and food-restricted Japanese quail chicks. In addition, we have measured the organ-specific expression patterns of mRNA species involved in growth and metabolism [insulin-like growth factor I (IGF-I), type II iodothyronine deiodinase (D2), TSHβ, thyroid hormone receptor α (TRα) and thyroid hormone receptor β (TRβ)], i.e. the thyroid hormone signalling pathway.

MATERIALS AND METHODS

Animal maintenance and experimental design

The present study was conducted using captive Japanese quails (*Coturnix japonica*, Temmick and Schlegel 1849). Fertilised eggs were purchased from a local distributer and hatched in our laboratory (incubator: type 180, America A/S, Thisted, Denmark). Chicks were removed from the incubator when they were 6–8 h old. When removed from the incubator the chicks were individually marked with flexible rings for identification (the rings were changed as the chicks grew bigger) and transferred to brooding boxes (105×95×25 cm) with sawdust bedding. Each box was supplied with an overhanging heating lamp. Chicks were fed a standard quail starter diet (protein content of 26%) and had free access to water throughout the experimental period. All birds received food *ad libitum* until 6 days of age. When the birds were 6 days old they were randomly divided into two treatment groups, one control group fed *ad libitum* and one experimental group receiving a weight-maintenance diet. The experimental birds were provided small amounts of food every three hours from 09:00 to 21:00 h to maintain a close to stable body mass. The control birds were sampled (see below) when they were 6, 8 or 11 days old. The experimental birds were either sampled at day 8, after 2 days of food restriction (2 days food-restricted group) or sampled at day 11, after 5 days of food restriction (5 days food-restricted group).

Metabolic measurements and dissection

RMR was measured as O_2-consumption rates using open flow-through respirometry. Measurements were done during the night in the dark, and the ambient temperature was kept at 35°C, which is within the thermoneutral zone for Japanese quail chicks (van der Ziel and Visser, 2001). Oxygen concentration in the effluent air was measured using a Servomex Xentra, type 4100, two channel oxygen analyser (Servomex Controls, Crowborough, England). Four birds were measured simultaneously in four black-enamelled metabolic chambers (1.5 l) using a flow rate of 400 ml min^{-1}. The measurement protocol is the same as used for zebra finches (*Taeniopygia guttata*) described in detail in Rønning et al. (Rønning et al., 2005).

Immediately after the metabolic measurements we collected blood samples (see below) and obtained biometrical measurements of wing, tarsus and skull (head plus bill). Then the birds were kept in individual cloth bags until they were randomly killed to collect tissue samples. The birds were cut open and the liver and the kidney were removed and weighed to the nearest 0.1 mg with a Sartorius digital scale (DWS, Elk Grove, IL, USA). Then a small piece of liver (>50 mg; apex of left lobe) and the left cerebral hemisphere of the brain were removed and immediately placed in test tubes and frozen in liquid nitrogen. The tissue samples were then stored at –70°C until further processed for mRNA analysis.

Blood analyses

Blood samples (~100 μl) were collected from the branchial vein using a heparinised capillary tube. The blood was centrifuged at 13,400 ***g*** for 3 min to separate the plasma from the blood cells. The red blood cells were stored frozen for later DNA sexing (Griffiths et al., 1998). The plasma was kept at –20°C until final T3 and T4 analyses. Total plasma concentrations of T3 and T4 were determined by radioimmunoassay at the Centre d'Étude Biologiques de Chizé, France, as detailed in Chastel et al. (Chastel et al., 2003) and Cherel et al. (Cherel et al., 2004). Only one assay for each hormone was performed and the intra-assay coefficients of variation were 4.8% for total T3 (N=7 replicates) and 3.7% for total T4 (N=7 replicates).

Quantitative PCR

Total RNA was purified from liver tissues homogenised in Trizol reagent according to manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). For brain, the left cerebral hemisphere was homogenised and an aliquot of 100 μl was used for total RNA isolation. The integrity of the RNA samples was verified by spectrophotometric analysis and formaldehyde agarose gel electrophoresis. Complementary DNA (cDNA) for the quantitative PCR (q-PCR) reactions were generated from 1 μg total RNA from all samples using a combination of random hexamer and poly-T primers from iScript cDNA Synthesis Kit, as described by the manufacturer (Bio-Rad, Hercules, CA, USA). The expressions of individual gene targets were analysed using the Mx3000 real-time PCR system (Stratagene, La Jolla, CA, USA). Every 25 μl DNA amplification reaction contained 12.5 μl of iTAQ^{TM}SYBR$^{®}$ Green Supermix with ROX (Bio-Rad), 1 μl of cDNA and 200 nmol l^{-1} of each forward and reverse primers. The three-step real-time PCR program included an enzyme activation step at 95°C (3 min) and 40 cycles of 95°C (15 s), 55–65°C for 15 s, depending on the primers used (see Table 1), and 72°C (30 s). Controls lacking cDNA template were included to determine the specificity of target cDNA amplification. Standard plots were generated for each target sequence using known amounts of plasmid containing the amplicon of interest. Cycle threshold (*Ct*) values were converted into initial target cDNA concentration using standard plots of *Ct versus* log DNA concentration (ng $μl^{-1}$). The criterion for using the standard curve is based on equal amplification efficiency with unknown samples and this is checked prior to extrapolating unknown samples to the standard curve. Data obtained from triplicate runs for target cDNA amplification were averaged and expressed as a percentage of the control samples. This absolute quantification method is a well-validated procedure in our laboratory, as we do not use the so-called housekeeping genes because of their parallel modulation pattern with experimental samples both in our laboratory (Arukwe, 2006) and elsewhere (Steele et al., 2002).

Statistical analyses

To perform analysis of variance (ANOVA) and analysis of covariance (ANCOVA) we used a general linear model (GLM) with

Table 1. Primer pair sequences used for real-time PCR, the amplicon size and annealing temperature used for quantitative PCR

Target gene	Primer sequence[1]		Amplicon size (nucleotides)	Annealing temperature (°C)
	Forward	Reverse		
IGF-I	ACAGGGTATGGATCCAGCAG	CATATCAGTGTGGCGCTGAG	159	57
TSHβ	TCTCTCCTCTTTGGCCTGAC	TGTGCACACGTTTTGAGACA	201	57
D2	GAAAATGTGCTGGTGGTGTG	GCTCCTTCAAAATTGCTTGC	196	57
TRα	GATGGAATTGCGGTGAATG	ACCCATCGTACTTGCAGGAG	301	57
TRβ	TGTTGGATGACAGCAAGAGG	TTTTGATCAGCTCCCATTCC	122	60

[1]Sequences are given in the 5′–3′ order. IGF-I, insulin-like growth factor I; TSHβ, thyroid-stimulating hormone β; D2, type II iodothyronine deiodinase, TRα, thyroid receptor α; TRβ, thyroid receptor β.

type III sum of squares. In models analysing RMR, body mass, organ mass, structural size and thyroid hormones the food treatment (treatment group) was entered as a three-level fixed factor (1=control, 2=2 days food-restricted, 3=5 days food-restricted). Differences between control and food-restricted birds on the same day were analysed using Student's *t*-tests. Due to relatively small sample sizes we did not adjust *P*-values in multiple comparisons (Nakagawa, 2004). Sex was not a significant factor in any model and was consequently not included in our analysis. RMR show allometric relationships with body mass and organ masses. Consequently, we linearised these variables by $\log_{10}$ transformation when analysing the effect of body mass and organ masses on RMR. To avoid a potential part-whole correlation we used body mass excluding organ mass when body mass and organ mass was included in the same model. The effect of food restriction on mRNA expression was analysed using five groups (1=day 6 control, 2=day 8 food *ad libitum*, 3=day 8 food-restricted, 4=day 11 food *ad libitum* and 5=day 11 food-restricted). Student's *t*-tests were used to compare *ad libitum* fed and food-restricted birds on the same day. When the mRNA data were not normally distributed we used a Mann–Whitney *U*-test for separate comparisons between the groups. Levels of significance were set at $P<0.05$. All statistical analyses were performed using SPSS v. 15.0 (SPSS, Chicago, IL, USA).

RESULTS

Body mass, organ masses and structural size

Eight and 11 days old experimental birds, subjected to 2 and 5 days of food restriction, had 25 and 41% lower body mass, respectively, compared with *ad libitum* fed chicks of the same age (Fig. 1). Both liver and kidney masses in the food-restricted birds were lower compared with *ad libitum* fed birds of the same age (Table 2). When controlling for the effect of body mass the effect of food treatment remained significant in the liver ($F_{2,63}=13.88$, $P<0.001$) but was only close to significance in the kidney ($F_{2,63}=2.99$, $P=0.057$).

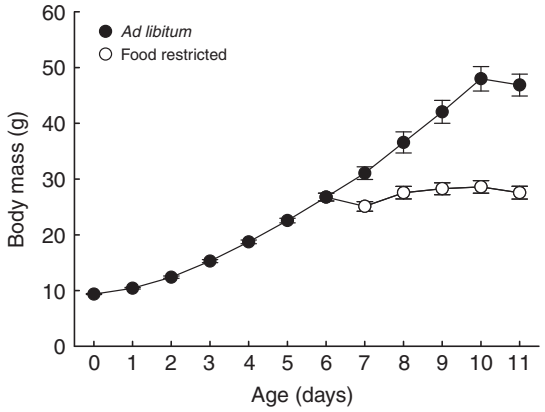

Fig. 1. Body mass growth of *ad libitum* fed (day 0–11) or food-restricted (day 6–11) Japanese quails. Body mass was measured in the morning each day and is represented as means ± s.e.m. The drop in body mass at day 11 is a consequence of all birds having stayed post-absorptive in the metabolic chamber before measurements were obtained.

Food restriction resulted in reduced growth of structural size, and the length of tarsus, skull and wing of birds subjected to food restriction for 5 days was substantially shorter than in the same aged control birds (Table 2). However, for a given body mass, the birds in the 5 days food-restricted group had significantly longer wing (39%), tarsus (6%) and skull (5%), compared with controls ($P\leq0.002$). Thus, the structural growth was not restrained to the same degree as body mass.

RMR

Food-restricted birds had a lower mass-dependent RMR ($ml\,O_2\,h^{-1}$) compared with controls of the same age (Fig. 2A). Resting metabolic rate was highly correlated to body mass ($F_{1,45}=484.8$, $P<0.001$, $R^2=0.92$) and scaled to body mass by the power of 0.83 (±0.04 s.e.m.) (Fig. 2B). The scaling between RMR and body mass did not differ between the treatment groups (treatment group × body mass interaction, not significant) (Fig. 2B). Pair-wise comparisons showed that the reduction in RMR was influenced by the length of the food restriction period, and for a given body mass the birds in the 2 days food-restricted group had 17% lower RMR whereas birds in the 5 days food-restricted group had 33% lower RMR than control birds (Fig. 2B, $P<0.001$).

Thyroid hormones

Food treatment during development produced a significant effect on the plasma T3 ($F_{2,64}=15.28$, $P<0.001$) and T4 ($F_{2,64}=15.08$, $P<0.001$) levels. Pair-wise comparisons between the control and experimental birds at different ages revealed that the food-restricted birds had significantly lower plasma concentrations of both thyroid hormones. The difference between control and experimental birds was significant already after 2 days of food restriction (Fig. 3A,B). Neither T3 nor T4 plasma concentrations differed significantly between different aged control birds (Fig. 3A,B).

Determinants of resting metabolic rate

The relationships between organ masses and plasma thyroid hormone levels, and RMR were analysed in separate models. Body mass and food treatment were significant determinants of RMR (see above) and were therefore included in the models. Neither the mass of the liver ($F_{1,44}=2.06$, $P=0.158$) nor kidney ($F_{1,44}=0.876$, $P=0.354$) explained a significant portion of the variation in RMR. There was a significantly positive relationship between plasma T3 and RMR ($F_{1,42}=10.155$, $P=0.003$, $R^2=0.195$) but the relationship differed between birds from the different treatment groups (treatment group × plasma T3 interaction, $F_{2,44}=5.570$, $P=0.007$). Parameter estimates

Table 2. Mean values (±s.e.m.) of liver and kidney mass (wet mass), and length of tarsus, skull and wing of *ad libitum* fed and food-restricted Japanese quails

	Day 6	Day 8		Day 11	
Parameter	(*N*=23)	*Ad libitum* (*N*=11)	2 days food-restricted (*N*=10)	*Ad libitum* (*N*=11)	5 days food-restricted (*N*=12)
Liver (g)	1.093 (0.050)	1.255 (0.054)	0.886 (0.048)***	1.652 (0.081)	0.966 (0.052)***
Kidney (g)	0.292 (0.013)	0.375 (0.025)	0.253 (0.012)***	0.562 (0.027)	0.329 (0.020)***
Tarsus (mm)	20.20 (0.34)	22.07 (0.68)	20.28 (0.31)*	25.12 (0.34)	22.52 (0.35)***
Skull (mm)	27.55 (0.20)	29.39 (0.30)	28.55 (0.32)	32.19 (0.29)	29.93 (0.29)***
Wing (mm)	33.14 (0.86)	42.60 (1.38)	37.10 (2.27)	61.80 (1.03)	54.50 (1.01)***

Asterisks indicate significant differences between same aged *ad libitum* fed and food-restricted birds. *P<0.05, ***P<0.001.

showed that there were no significant relationships between plasma T3 and RMR in the control birds (R^2=0.005, P=0.661) or in the 2 days food-restricted birds (R^2=0.036, P=0.216) whereas in the 5 days food-restricted birds, there was a significant positive relationship (R^2=0.212, P=0.002). As opposed to T3, none of the variation in RMR was explained by the plasma T4 concentration ($F_{1,44}$=0.121, P=0.729).

IGF-I, TSHβ, D2 and TRα and β mRNA expression in the brain and liver

In the brain, no differences in IGF-I mRNA were observed between food-restricted and *ad libitum* fed birds (Fig. 4A). For the liver, which is the main contributor to circulating IGF-I, there was a 5-fold increase in the IGF-I mRNA level from day 6 to 8 in the *ad libitum* fed birds. During the same time period, IGF-I mRNA levels in the experimental birds showed a reduction, resulting in a significant difference between *ad libitum* fed and food-restricted birds at 8 days of age (Fig. 4A).

Food restriction tended to decrease TSHβ mRNA expression both in the brain and liver but the treatment effect was not significant (Fig. 4B). There were no differences in the expression of D2 in the liver between same aged *ad libitum* fed and experimental birds (Fig. 4C). However, food treatment tended to influence the expression pattern of D2 mRNA in the brain. Contrary to the *ad libitum* fed birds, the D2 transcript levels in the experimental birds apparently increased with age (Fig. 4C). However, the difference in

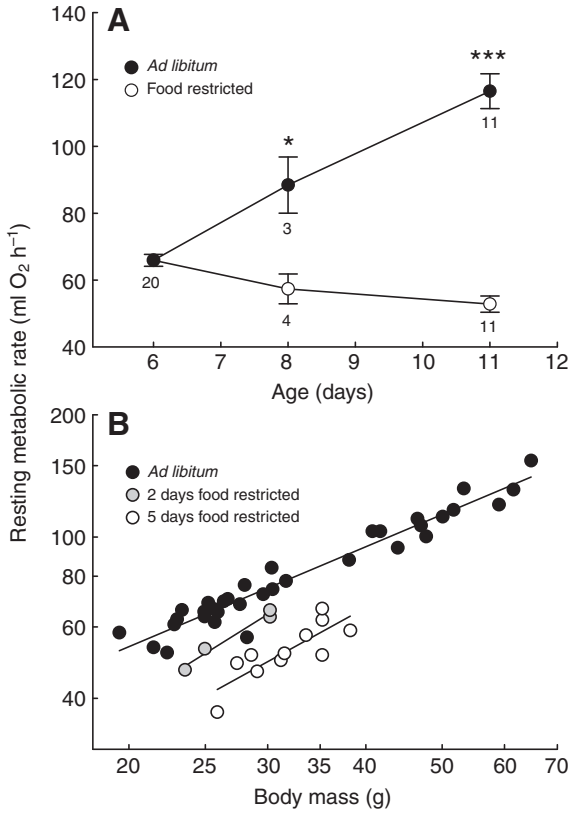

Fig. 2. (A) Resting metabolic rate (RMR) (means ± s.e.m.) in different aged *ad libitum* fed and food-restricted Japanese quails. Asterisks indicate a difference between *ad libitum* and food-restricted birds on the same day (*P<0.05, ***P<0.001). Numbers below whiskers represent sample sizes. (B) RMR as a function of body mass. Linear regression lines are shown for each treatment group. The axes are log-scaled.

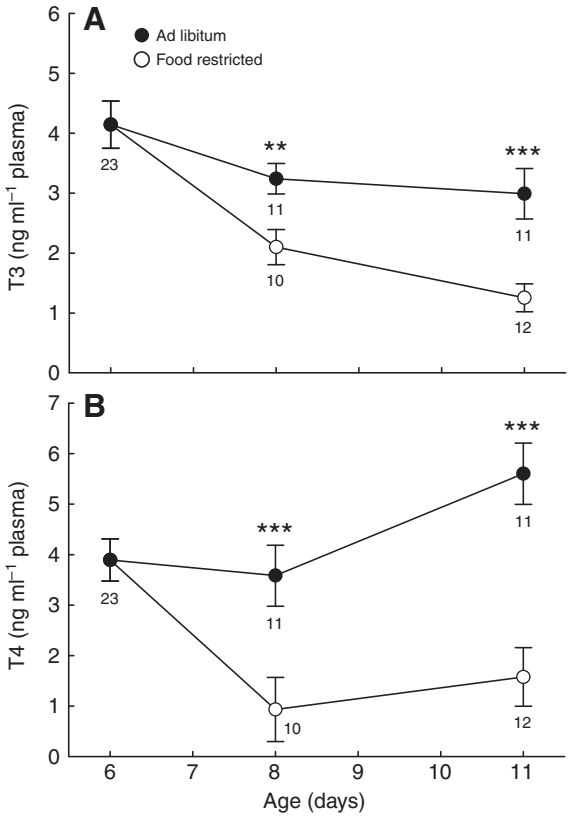

Fig. 3. Total plasma concentration (means ± s.e.m.) of (A) 3,3′,5-triiodo-L-thyronine (T3) and (B) 3,5,3′,5′-tetraiodothyronine (T4) in *ad libitum* fed and food-restricted Japanese quails. Asterisks denote a difference between *ad libitum* and food-restricted birds on the same day (**P<0.01, ***P<0.001). Numbers below whiskers represent sample sizes.

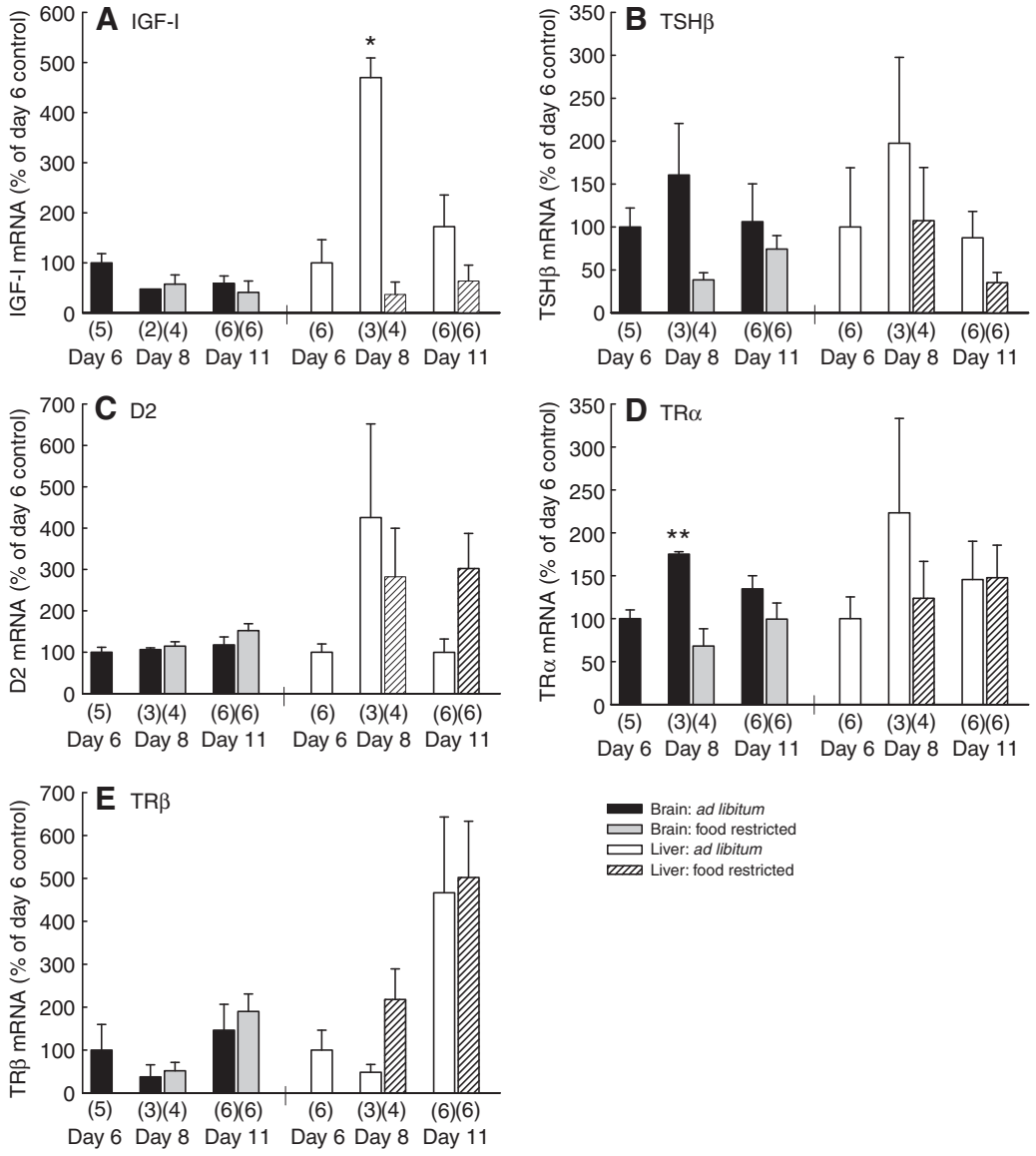

Fig. 4. Gene expression of (A) insulin-like growth factor I (IGF-I), (B) thyroid-stimulating hormone β(TSHβ), (C) type II iodothyronine deiodinase (D2), (D) thyroid receptor α(TRα) and (E) thyroid receptor β(TRβ) mRNA in the brain and liver from *ad libitum* fed and food-restricted Japanese quails at different ages. The data are given as a percentage of day 6 control group (± s.e.m.). Asterisks over the columns denote a significant difference between *ad libitum* fed and food-restricted chicks on the same day (**P*<0.05, ***P*<0.01). Numbers below columns represent sample sizes.

D2 mRNA expression between same aged *ad libitum* fed and food-restricted birds failed to reach significance. For TRα, a parallel expression pattern was observed between liver and brain (Fig. 3D). However, a significant effect of the food treatment was observed only in the brain where the 8 days old *ad libitum* fed birds showed higher TRα mRNA levels than the same aged experimental birds (Fig. 4D). Although the expression of TRβ, the other thyroid-hormone receptor isoform investigated, tended to be higher in both the brain and the liver of the food-restricted birds, no significant differences in expression between same aged treatment groups were found (Fig. 4E).

DISCUSSION

The objective of the present study was to study morphological and physiological responses in young Japanese quails subjected to food restriction. By comparing *ad libitum* fed and food-restricted birds, we were able to study developmental plasticity elicited by undernourishment. In general, our data showed that the weight-maintenance diet resulted in a decreased growth of structural features. In addition, RMR was substantially reduced in birds that experienced food restriction. Furthermore, food restriction produced significant effects on circulating thyroid hormones and differentially affected mRNA species in the thyroid hormone signalling pathway that are believed to influence growth and metabolism.

Growth

In our present study, dietary effects on both structural growth and internal organs were observed. The structural growth slowed down in the experimental birds and a significant difference in structural size was observed already after 2 days of food restriction. However, for a given body mass, the 5 days food-restricted birds were structurally bigger then *ad libitum* fed birds, indicating that structural growth was not restrained to the same degree as body mass. Such a response is also shown for Peking ducklings *Anas platyrhyncos* (Moe et al., 2005b) and European shag nestlings *Phalacrocorax aristotelis* (Moe at al., 2004). Thus, some part of the limited available energy was devoted to structural growth at the expense of an increase in body mass. The liver and kidney responded differently to food restriction. Food-restricted birds had a lower kidney mass compared with controls but when controlling for the effect of body mass the effect of food restriction was only close to significance. The liver mass, however, was highly reduced relative to body mass, both in

the 2 and 5 days food-restricted birds. These findings indicate that energy from nutrients was potentially scavenged from the liver to support other important biological processes in birds undergoing food restriction.

IGFs are believed to be important for normal growth and development and influence, among other things, glucose and amino acid uptake, DNA and protein synthesis and proliferation of cells (McMurtry et al., 1997). Plasma IGF-I in precocial species such as the Japanese quail is known to peak during the post-natal period of rapid growth (Schew et al., 1996). In the liver of *ad libitum* fed quails, we found increased levels of IGF-I mRNA from day 6 to day 8 of age, possibly representing such a post-natal peak in IGF-I. Although we did not measure IGF-I levels in the plasma, earlier studies on precocial species have found that hepatic mRNA expression of IGF-I correlated with plasma concentration of IGF-I (Rosselot et al., 1995; Kita et al., 1998). The increase in IGF-I expression from day 6 to day 8 was not observed in the experimental birds, where a substantially lower liver IGF-I expression was observed in the 2 days food-restricted birds compared with birds fed *ad libitum*. A decrease in hepatic IGF-I expression seems to be a common physiological reaction to underfeeding, at least in domestic fowl (Beccavin et al., 2001; Kita et al., 2005). This effect may differ between tissues, e.g. IGF-I expression in the brain in our present study and the ovaries in a study of broiler breeder hens by Heck et al. (Heck et al., 2003) did not change as a result of food restriction. However, the liver is believed to be the major contributor to plasma IGF-I (Rosselot et al., 1995), indicating that several tissues could be affected through endocrine action by an alteration in hepatic IGF-I expression. Given the fact that liver is the main source of circulating IGF-I, it is tempting to interpret the lower hepatic mRNA expression found in our food-restricted birds compared with *ad libitum* fed birds as a mechanism to reduce energy devoted to growth. However, results from studies on domestic fowl on the relationship between post-hatch growth and circulating IGF-I are somewhat inconclusive. For example, experiments in poultry with infusion of IGF-I have been shown to enhance growth (Tomas et al., 1998) whereas in other studies no effect of exogenous IGF-I treatment was reported (Huybrechts et al., 1992). Elsewhere, in studies that compared different lines of chickens with high and low growth rates, plasma IGF-I and hepatic IGF-I mRNA showed higher levels in birds with high growth rates (Beccavin et al., 2001; Duclos, 2005). Other studies that used similar models did not report any positive relationships between plasma IGF-I and growth rate (Goddard et al., 1988; McMurtry et al., 1997). IGFs have repeatedly been shown to influence glucose and lipid metabolism, and it has therefore been suggested that IGFs may be more related to intermediary metabolism than to growth *per se* in birds (McMurtry et al., 1997). Thus, the lower hepatic IGF-I expression observed in food-restricted birds in our present study may contribute to the lower resting metabolic rate found in these birds and also to potential effects on growth.

Metabolism

RMR in the Japanese quail chicks was strongly affected by food treatment. A lower RMR compared with *ad libitum* fed subjects was observed already after 2 days of food restriction and the effect was further increased after 5 days. A similar energy saving mechanism during low food availability at developmental stages has previously been reported in Japanese quails (Schew, 1995) as well as in other precocial birds species such as meat-type chickens (Zubair and Leeson, 1994) and Peking ducklings (Moe et al., 2005b). Reducing the amount of energy used for maintenance makes more energy available for other important tasks during development. Hence, a reduction in metabolic processes has been interpreted as an adaptational response that enhances survival during poor feeding conditions (Schew and Ricklefs, 1998). Young birds experiencing a shortage of food are likely to lower their body temperature (Moe et al., 2004; Moe et al., 2005a; Moe et al., 2005b). Such hypothermia is associated with a reduced metabolic rate. We did not measure body temperature in this study but Schew showed that food-restricted young Japanese quails lowered their body temperature (Schew, 1995). Thus, hypothermia is likely to be one factor associated with the reduced RMR found in the food-restricted birds in our present study.

Specific metabolic rate varies substantially between tissues, and organs that have a high specific metabolic rate such as the liver and kidney are expected to contribute disproportionately to whole-body RMR (Rolfe and Brown, 1997). For a given body mass, the experimental birds had lower liver masses compared with *ad libitum* fed birds. Thus, liver mass may have contributed to the lower RMR found in the food-restricted birds. However, liver mass did not explain a significant portion of the variation in RMR within the treatment groups, indicating that the effect of liver mass on RMR was probably small. Kidney mass was maintained with respect to body mass irrespective of food treatment (the effect of food treatment was only close to significance) and did not correlate to RMR. However, metabolic reduction in black legged kittiwakes (*Rissa tridactyla*) has been shown to involve adjustments of intrinsic metabolism in the kidney (Rønning et al., 2008), indicating that these organs could contribute to changes in RMR irrespective of relative mass change.

In homeothermic animals, thyroid hormones are involved in regulating basal energy expenditure. Thus, the lower RMR in the food-restricted birds in our present study could be mediated by changes in plasma thyroid levels, especially T3 as this is considered the most functionally active form (Decuypere et al., 2005). As in previous studies of Japanese quails, we found that plasma T3 levels decreased as a response to food restriction (Schew et al., 1996; Kobayashi and Ishii, 2002), indicating that plasma T3 was involved in slowing metabolic rate in food-restricted birds. In the *ad libitum* fed birds there was no correlation between plasma T3 concentration and RMR. However, in the experimental birds, T3 was positively correlated to RMR, although only significantly so in the 5 days food-restricted group. These results indicate that plasma T3 may be a more important regulator of RMR during periods of suppressed metabolism (e.g. during food restriction) than during periods when metabolism is regulated at a ‘normal’ level. Similar to T3, plasma T4 level was also lowest in the food-restricted birds. Contrary to T3, plasma T4 did not contribute in explaining RMR variation, which is not surprising as T4 is less potent modulator of energy metabolism (Decuypere et al., 2005).

Thyroid hormones play crucial roles in brain functioning and during neural development (Bernal, 2002). Thus, ensuring that T3 levels remain within a narrow limit should be especially important in the brain compared with other tissues. Available evidence suggests that the chicken is able to maintain a local systemic T3 content in the brain that is more or less independent of plasma level (Reyns et al., 2005; Rudas et al., 2005). One mechanism that has been suggested to be important in this local regulation is the conversion of T4 to T3 through deiodinase activities (Rudas et al., 2005). In the present study, we measured the mRNA expression for D2, an enzyme that is important for intracellular production of receptor active T3 (Gereben et al., 2008). If plasma concentration

of thyroid hormones decreases, an increased expression of D2 could serve as a protective mechanism to increase the conversion of T4 to T3 in order to maintain a critical T3 level in the brain. Thus, as thyroid hormone levels decreased with the length of food restriction in our present study we expected a paralleled increased D2 expression in the brain. An expression pattern with increased D2 expression as a response to food restriction was indeed observed, although the difference between same aged food restricted and *ad libitum* fed birds did not reach significance. Furthermore, there was an inverse relationship between individual plasma T3 and brain D2 mRNA levels (Pearson's R=–0.587, P=0.003, data not shown). The relationship between plasma T4 and brain D2 mRNA expression was not significant, suggesting that T3 is the most important thyroid hormone when it comes to regulating brain D2 expression in Japanese quails. There were no significant correlations between plasma levels of either T3 or T4 and hepatic D2 expression, indicating that D2 in the liver is not as strictly regulated by plasma thyroid hormones as in the brain.

T3 binds to both isotypes of thyroid hormone receptors (TRs), and several T3-mediated effects are regulated through the role of TRs as hormone inducible transcription factors for their target genes (Yen, 2001). Consequently, the abundance of these hormone receptors in a given tissue highly influences the local effect of T3. In the liver, we did not find any significant differences in mRNA expression of either TRα or TRβ between same aged *ad libitum* fed and experimental birds. TRβ mRNA levels tended to be higher in food-restricted birds but because TRα was by far the predominant receptor expressed in the liver, these adjustments in TRβ expression were probably not very effective to counteract effects of low plasma T3 concentrations. In the brain, we did not find any effect of food restriction on TRβ expression. TRα, by contrast, was significantly lower in the 2 days food-restricted birds compared with the same aged *ad libitum* fed birds. An elevation in the content of both TRα and TRβ in the telencephalon in response to low plasma thyroid hormone levels has previously been reported in hypothyroid chicken (Gereben et al., 1998). One reason for the absence of an increased TRs expression in the food-restricted birds could be that brain T3 levels were regulated, i.e. the concentrations of plasma thyroid hormones were not low enough to influence brain T3 levels. However, we cannot rule out the possibility that TRs mRNA expression in some brain areas actually was affected by food restriction but that this was masked because we used homogenised brain tissue in our study.

In summary, we showed herein that Japanese quail chicks subjected to food restriction exhibited substantial energy saving mechanisms by reducing their structural growth as well as their metabolism. Plasma thyroid hormone levels decreased in food-restricted birds. Interestingly, circulating T3 was observed to be a putatively, strong predictor of RMR variation in the 5 days food-restricted birds showing the strongest reduction in metabolism. With respect to effects on mRNA expression levels in the thyroid hormone signalling pathway, the reduction in thyroid hormone levels paralleled an apparent increase in brain D2 expression, indicating that developing Japanese quails are able to carry out counteracting responses to prevent hypothyroid conditions in the brain. Furthermore, reduced IGF-I expression in the liver of food-restricted birds suggests that a downregulation of hepatic IGF-I expression is a putative mechanism employed by developing Japanese quails to slow growth and metabolism during period of food restriction. Thus, the Japanese quails showed changes in gene expression of factors that affect physiological changes due to food restriction with subsequent effects on growth and metabolism.

LIST OF ABBREVIATIONS

Ct	cycle threshold
D2	type II iodothyronine deiodinase
IGF-I	insulin-like growth factor I
RMR	resting metabolic rate
TRα	thyroid hormone receptor α
TRβ	thyroid hormone receptor β
TSHβ	thyroid-stimulating hormone β
T3	3,3′,5-triiodo-L-thyronine
T4	3,5,3′,5′-tetraiodothyronine

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