A role for thyroid hormones in the regulation of diet-induced thermogenesis in birds

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The possible involvement of thyroid hormones in avian diet-induced thermogenesis (DIT) was investigated in two lines of cockerels divergently selected for high (R−) or low (R+) food efficiency. For a given body weight, R+ cockerels exhibited a higher food intake than R− cockerels (+49 to +76 %) and increased DIT (+25 %). Plasma thyroxine (T_4) levels did not differ between lines whatever the feeding status of the birds. Plasma 3,5,3′-triiodothyronine (T_3) level was lower in fasted R+ than in fasted R− cockerels while the opposite was observed after a meal. Iopanic acid injections reduced both plasma T_3 concentrations and heat production to the same levels in both lines. Hepatic 5′-deiodinase activity measured with an exogenous sulfhydryl group (dithiothreitol) did not differ between lines, but when the sulfhydryl group was omitted, the activity was higher in R+ than in R− birds (90 *v.* 42 pmol T_3/min per liver). T_3-binding capacity of isolated hepatic nuclei was higher (+76 %) in R+ than in R− birds. Long-term or acute pair-feeding of R+ cockerels to the level of R− controls did not alter these results. The present results suggest that T_3, mainly originating from peripheral conversion of T_4 to T_3, is involved in DIT in the R+ line. Availability of endogenous sulfhydryl groups appears to play an important part in the modulation of hepatic deiodinase activity. The higher concentration of nuclear T_3 receptors may further increase the effects of the hormone, suggesting a major role of thyroid hormones associated with catecholamines in the stimulation of avian DIT. The underlying thermogenic mechanisms remain to be elucidated.

Diet-induced thermogenesis: Thyroid hormones: Birds

In poultry production, two-thirds of the total cost of meat or egg production is expenditure on feed. Hence, the improvement of feed efficiency (the reduction of food intake for a given level of production) has long been a major preoccupation of breeders and geneticists. In laying hens, an important part of the variation in food consumption can be explained by the egg mass, the body weight and its variation (Byerly *et al.* 1980; Fairfull & Chambers, 1984). The remaining variation, which is referred to as residual food intake (the 'R' criterion), may be used in selection for feed efficiency. R is defined as the difference between the individually observed and predicted (by regression between food intake and the egg mass, the body weight and its variation) food intakes. Two experimental lines have been obtained by divergent selection for high (R+) or low (R−) residual food intake (Bordas & Mérat, 1984; Bordas *et al.* 1992). The observed food intakes differ by 40 % for males of the same body weight and by 30 % for females for the same body weight and level of egg production after fourteen generations of selection. Body composition differs between lines, but surprisingly, R+ birds are leaner than R− birds (Zein-El-Dein *et al.* 1985;

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Tixier *et al.* 1988; El-Kazzi *et al.* 1995). Using indirect calorimetry, Géraert *et al.* (1991) and Gabarrou & Géraert (1994*a,b*) demonstrated that the difference in energy intake was explained by enhanced heat production in the R+ compared with the R− line. While the BMR and activity levels do not differ between these lines, this difference in heat production originates in an enhanced heat increment of food or diet-induced thermogenesis (DIT) whose regulatory component (Gabarrou *et al.* 1998) is mainly under β-adrenergic control in the R+ line (Gabarrou & Géraert 1994*a,b*).

In rats, hyperphagia induced by a cafeteria meal is also accompanied by a large increase in metabolic rate due to a regulatory DIT, under β-adrenergic control, and increased plasma 3,5,3′-triiodothyronine (T_3) concentration (Rothwell & Stock, 1979). In human subjects, Danforth *et al.* (1979) also suggest that overfeeding induces increased plasma T_3 concentration while plasma thyroxine (T_4) level remains unchanged. The possibility therefore exists that thyroid hormones, mainly T_3 are involved in DIT. T_3 may originate either from the thyroid gland or from peripheral deiodination of T_4 to T_3. At the cellular level, T_3 acts mainly via nuclear receptors, whose concentration is important in determining the cellular response (Lazar, 1993).

The present experiment was performed to investigate whether thyroid hormones control the energy balance of R+ and R− adult cockerels. Plasma T_3 and T_4 concentrations were determined in both lines, either in the fed state or after a short-term fast. In order to compare R+ and R− lines for a given energy intake level, one group of R+ birds was restricted for 10 weeks to the energy intake of R− cockerels. A second group of R+ birds was tube-fed once with the same amount of food as R− birds. To investigate the control of plasma T_3 concentration by peripheral deiodinase, the effect of iopanoic acid on energy expenditure and on plasma T_3 and T_4 concentrations was investigated *in vivo* and the activity of hepatic 5′-deiodinase was also determined. The concentration of T_3 binding sites was also estimated on isolated hepatic nuclei.

MATERIALS AND METHODS

Animals and diet

Adult cockerels of the twentieth generation of the R+ and R− lines (Bordas *et al.* 1992), aged 36–50 weeks at the time of the experiments, were reared in individual battery cages. They were fed on a standard complete diet containing 12·84 MJ metabolizable energy (ME) and 126 g crude protein/kg. Birds had free access to water. Ambient temperature was maintained at 20° in the thermoneutral zone of the cockerels. The lighting programme was 14 h light : 10 h dark.

Expt 1

To enable comparison of R+ and R− cockerels for a given level of energy intake, three groups of seven birds were selected from the two populations of R+ and R− birds. Seven cockerels of each line had free access to food (R + F and R−F) and food intake was measured daily. Seven R+ birds (R + P) were individually selected for the corresponding body weight of an R−F cockerel. They were pair-fed daily with the food intake of the R−F cockerel of the same body weight for 10 weeks. Birds were weighed before and after the restriction period. Blood was collected before the restriction period after a 16 h fast, and after the restriction period when re-fed. Blood was collected via a wing vein using a heparinized syringe. Plasma was separated and stored at −20° until determination of T_3 and T_4 by radioimmunoassay as described previously by Gabarrou (1996). At the end of

the experiment, birds were bled and killed by cervical dislocation 4 h after the onset of light; thus they had free access to food for 4 h before death. The liver was dissected rapidly, frozen in liquid N_2 and kept at $-70°$ until analysis.

5′-Deiodinase activity was determined as described previously in chickens by Kuhn *et al.* (1987) with slight modifications. In brief, 1 g liver was homogenized in four volumes of Tris–sucrose–EDTA buffer (0·05 M-Tris-HCl, 0·25 M-sucrose, 0·005 M-EDTA, pH 7·2) and centrifuged at 10 000 ***g*** for 30 min at 4°. Duplicate 0·2 ml portions of the supernatant fraction were immediately incubated in 0·4 ml Tris–sucrose–EDTA buffer, 0·1 ml 12·8 μM-T_4 solution with or without 0·1 ml 20 mM-dithiothreitol (DTT). One series of tubes was kept on ice and 2 ml ice-cold 950 ml/l ethanol and 0·2 ml 8-anilino-naphthalenesulfonic acid (ANS; 1 mg/ml) were added. Other tubes were incubated for 30 min at 37°. The reactions were stopped by cooling the tubes on ice and adding 2 ml ice-cold ethanol and 0·2 ml ANS. The tubes were centrifuged at 3000 ***g*** for 20 min. The supernatant fractions were diluted threefold with buffer and assayed for total T_3 using a Coat-a-Count RIA kit (Diagnostics Products Corporation, Los Angeles, CA, USA). Protein concentration of the liver homogenates was determined by the bicinchoninic acid method and ranged between 25 and 30 mg/ml.

Expt 2

To enable a comparison of R+ and R− cockerels for a given level of energy intake, three groups of five birds were selected from the two populations of R+ and R− birds. Five cockerels of each line were fasted for 16 h and tube-fed with 50 % of their *ad libitum* food intake: 67 and 43 g food for R + F and R − F groups respectively. Five R+ birds (R + P) were also fasted for 16 h but tube-fed with only 43 g food (as R − F group, see Table 1).

Birds were weighed after the fast and before tube-feeding. Blood was collected after the fast and 4 h after tube-feeding. Blood was processed as in Expt 1. Birds were killed as described earlier 4 h after tube-feeding and the liver was dissected rapidly and frozen as in Expt 1. Deiodinase activity was determined as described earlier. Liver T_3 receptor level was determined as described by Dauncey *et al.* (1992) with some modifications. Briefly, liver samples were homogenized in buffer A containing: 0·2 mM-K_2HPO_4, 0·6 mM-KH_2PO_4, 250 mM-sucrose and 1 mM-$MgCl_2$ (pH 6·8), with the addition of 1 ml Triton X-100/l. The homogenates were then filtered through a mesh of 35 μm pore size. The filtrate was centrifuged at 750 g_{max} for 10 min and the pellet was washed twice in buffer A. The final pellet was layered over buffer B containing: 3·5 mM-K_2HPO_4, 2·3 M-sucrose and 1 mM-$MgCl_2$ (pH 6·8), and centrifuged at 70 000 g_{max} for 50 min. The pelleted nuclei were washed in buffer C containing: 0·2 mM-K_2HPO_4, 0·6 M-KH_2PO_4, 320 mM-sucrose and 1 mM-$MgCl_2$ and 1 mM-$CaCl_2$ (pH 6·8). This appeared to be an essential step to avoid aggregation of nuclei. They were finally suspended in buffer D containing: 10 mM-Tris, 320 mM-sucrose, 3·2 mM-$MgCl_2$, and 5 mM-DTT (pH 7·4). All procedures were carried out at 4°.

The DNA content of the nuclear extract was determined by the method of Labarca & Paigen (1980). Portions (200 μl) of the nuclear suspension containing 30 μg DNA were incubated in duplicate for 30 min at 37° with 100 μl ^{125}I-labelled T_3 (NEN, Life Science Products, Boston, USA) and increasing concentrations of unlabelled T_3 in buffer D. Tubes used to measure non-specific binding contained a 100-fold excess of unlabelled T_3. The nuclei were then centrifuged at 3500 ***g*** for 10 min. The pellet was washed twice in buffer D containing 10 ml Triton X-100/l and its radioactivity measured on a gamma counter (Auto gamma 5110, Packard, Warrenville, USA). Sufficient DNA was obtained from 2–3 g liver.

Liver samples of one cockerel from each group were always analysed within the same nuclear isolation and assay, because of possible variations between assays. Values for specific maximal T_3 binding capacity (B_{max}) and the dissociation constant for specific T_3 receptors (K_d) were obtained from Scatchard plots of bound/free *v.* bound after correction for non-specific binding.

Expt 3

Birds of the two lines had free access to food and water. Before the experiment, birds were individually weighed. Food intake was determined daily. Nine cockerels of each line were injected subcutaneously with iopanoic acid (50 mg/kg) and nine control cockerels of both lines were similarly injected with saline solution (9 g NaCl/l) as described by Decuypere *et al.* (1980). Birds received one subcutaneous injection per day in the morning for 2 d. Before the first injection, blood was collected and processed as in Expt 1. During the 4 h following the second injection birds had free access to food. They were then transferred to individual respiratory chambers to measure their energy balance for 4 h. To reduce activity during heat production measurement, birds had no food, no water and no light. After the 4 h period of heat production analysis, blood was collected and analysed as in Expt 1. At the same time, rectal and comb temperatures were determined using thermocouples (N93722 and N93713, Bioblock Scientific, France) previously calibrated against a Hg thermometer. Activity was measured using a Doppler radar system. O_2 consumption and CO_2 production were measured using an automated indirect multi-calorimetry system (Géraert, 1990). Heat production (HP) was calculated as:

$$HP\ (kJ) = 16{\cdot}18\,O_2\ (litres) + 5{\cdot}02\,CO_2\ (litres)\ (Romijn\ \&\ Lokhorst,\ 1961).$$

HP was measured 5 times/min over a 4 h period.

Statistical analysis

Data were analysed using ANOVA followed by a *post hoc* test for between-group differences. $P < 0{\cdot}05$ was considered significant.

RESULTS

Expts 1 and 2

The results for the residual food consumption for the two lines of cockerels showed clear differences and R+ birds ate much more than predicted by the null hypothesis. For a given body weight, R + F cockerels had a higher food intake (+40 %) than R−F cockerels (Table 1). When pair-fed for a long period of time (10 weeks), R + P birds lost weight (−10 %). Based on the usual food intake R+ and R− birds (134 and 86 g/d respectively), Expt 2 consisted of tube-feeding the cockerels once, with 50 % of this daily intake.

When subjected to a fast, R+ birds exhibited a lower plasma T_3 concentration than R− birds (Table 2). On the other hand, *ad libitum*-fed R+ birds exhibited a higher plasma T_3 concentration than R− birds. A similar but non-significant trend was observed in tube-fed birds. Plasma T_3 concentrations of R+ cockerels tended to decrease slightly after a long period of food restriction, but were not affected by the quantity of food given acutely by tube-feeding. In *ad libitum*-fed birds, no difference between lines was observed in plasma T_4 concentration. Long-term food restriction did not affect plasma T_4 concentration, whereas fasting increased it significantly in both lines.

Table 1. *Expts 1 and 2. Body weight, food intake and residual food consumption (RFC†) of cockerels divergently selected for high (R−) or low (R+) food efficiency*

(Mean values with their standard errors)

	R+F‡		R+P‡		R−F‡		
	Mean	SEM	Mean	SEM	Mean	SEM	*P*
Expt 1 (*n* 7)							
Body wt before restriction (g)	3333[a]	107	3414[a]	133	3412[a]	124	NS
Body wt after restriction (g)	3443[a]	94	3070[b]	114	3548[a]	126	*
Food intake (g/d)	113[a]	4	76[b]	3	76[b]	3	**
RFC (g/week)	149[a]	28	152[a]	21	−138[b]	45	***
Expt 2 (*n* 5)							
Body wt (g)	3433[a]	273	3180[a]	131	3464[a]	162	NS
Food intake (g/d)	67[a]	4	43[b]	3	43[b]	2	***
RFC (g/week)	181[a]	22	175[a]	9	−227[b]	42	***

[a,b] Mean values within a row with unlike superscript letters were significantly different: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

† Difference between observed food consumption (FC) and FC predicted by the regression: FC = a × body weight + b × body-weight gain + c.

‡ R+F and R−F were fed with their *ad libitum* food intake; R + P were pair-fed with R−F.

Hepatic 5′-deiodinase activity was measured in the presence or absence of DTT as an exogenous provider of essential sulfhydryl-groups (Table 3) since the thiol dependence of 5′-deiodinase is well established (Visser *et al.* 1976). In the presence of DTT, no difference between lines was observed whatever the level of food intake. When no DTT was added, deiodinase activity was reduced but to a lower extent in the R+ than in the R− line. Long-

Table 2. *Expts 1 and 2. Plasma triiodothyronine (T_3) and thyroxine (T_4) levels of cockerels divergently selected for high (R−) or low (R+) food efficiency*

(Mean values with their standard errors)

	R+F†		R+P†		R−F†		
	Mean	SEM	Mean	SEM	Mean	SEM	*P*
Plasma T_3 concentration (nmol/l)							
Expt 1 (*n* 7)							
Fasting	0·93[a]	0·18	0·91[a]	0·06	1·53[b]	0·23	*
Voluntarily fed	2·44[a]	0·24	1·76[ab]	0·14	1·39[b]	0·07	**
Expt 2 (*n* 5)							
Fasting	1·04[a]	0·07	1·06[a]	0·14	1·68[b]	0·14	*
Tube-fed	2·64[a]	0·25	2·62[a]	0·21	1·94[b]	0·15	*P* = 0·1
Plasma T_4 concentration (nmol/l)							
Expt 1 (*n* 7)							
Fasting	45·2[a]	2·7	55·2[a]	4·0	47·1[a]	4·5	NS
Voluntarily fed	26·2[a]	2·0	32·0[a]	3·0	26·0[a]	2·1	NS
Expt 2 (*n* 5)							
Fasting	35·9[a]	4·3	39·5[a]	6·7	31·0[a]	2·4	NS
Tube-fed	16·5[a]	3·2	19·2[a]	3·4	17·6[a]	2·9	NS

[a,b] Mean values within a row not sharing a common superscript letter were significantly different: $^{*}P < 0.05$, $^{**}P < 0.01$.

† R+F and R−F were fed at their *ad libitum* food intake level; R+P were pair-fed with R−F.

Table 3. *Expts 1 and 2. Hepatic deiiodinase activity, measured in the presence or absence of dithiothreitol (DTT) as an exogenous provider of sulfhydryl groups, in cockerels divergently selected for high (R−) or low (R+) food efficiency*

(Mean values with their standard errors)

	R+F†		R+P†		R−F†		
	Mean	SEM	Mean	SEM	Mean	SEM	*P*
With DTT							
Expt 1 (*n* 7)							
pmol T_3/min per g protein	55·1[a]	7·9	61·0[a]	5·4	62·2[a]	9·3	NS
pmol T_3/min per liver	233[a]	27	237[a]	29	325[a]	42	NS
Expt 2 (*n* 5)							
pmol T_3/min per g protein	62·1[a]	11·6	50·8[a]	3·3	72·8[a]	11·3	NS
pmol T_3/min per liver	414[a]	54	320[a]	55	326[a]	47	NS
Without DTT							
Expt 1 (*n* 7)							
pmol T_3/min per g protein	20·4[a]	2·5	16·7[a]	1·7	8·1[b]	2·2	*
pmol T_3/min per liver	90[a]	9	71[a]	16	42[b]	13	*
Expt 2 (*n* 5)							
pmol T_3/min per g protein	15·6[a]	1·9	18·3[ab]	5·8	7·6[b]	1·5	*
pmol T_3/min per liver	110[a]	16	99[ab]	28	36[b]	7	*

[a,b] Mean values within a row not sharing a common superscript letter were significantly different: $^*P < 0.05$.
† R+F and R−F were fed at their *ad libitum* food intake level; R+P were pair-fed with R−F.

term food restriction did not significantly reduce deiodinase activity but acute restriction coupled with tube-feeding increased the variability in deiodinase activity. When calculated on a by-liver basis, deiodinase activity without DTT was nearly threefold higher in R+ than in the R− cockerels.

Scatchard analysis of T_3 binding to isolated hepatic nuclei indicated one population of binding sites. An estimation of B_{max} per unit weight of DNA for hepatic nuclear T_3 receptors was determined. B_{max} values were significantly higher in R+ than in the R− cockerels (1·28 (SE 0·31) and 1·32 (SE 0·13) for R+F and R+P respectively *v.* 0·74 (SE 0·13) for R−F). Acute pair-feeding to the level of R− birds did not alter hepatic B_{max} values in R+ cockerels. Estimates of the T_3 receptor dissociation constant (K_d) were similar in the three groups of cockerels and averaged 3·5 (SE 0·4) nM.

Expt 3

As in the first series of cockerels used in Expts 1 and 2, R+ birds ate more (+76 %) than R− birds for a similar body weight (Table 4). Heat production was higher in the control R+ than in the control R− cockerels (+25 %). No differences in physical activity or RQ were observed between lines. Although rectal temperature did not differ significantly between lines, comb temperature was higher in the R+ than in the R− line. Iopanoic acid did not significantly affect food intake in either line, but reduced heat production and comb temperature in the R+ line to the levels measured in the R− line.

As in Expt 1, there was a higher plasma T_3 concentration in R+ than in R− birds, but no differences in plasma T_4 concentration (Table 5). Iopanoic acid reduced plasma T_3 concentration in both lines but to a lower extent in R− than in R+ cockerels (−0·87 *v.* −1·49 μmol/l). Conversely, iopanoic acid increased plasma T_4 concentration in both lines, but to a lower extent in R− than in R+ cockerels (+45·8 *v.* +61·6 μmol/l).

Table 4. *Expt 3. Measures of energy balance in cockerels divergently selected for high (R−) or low (R+) food efficiency, injected with iopanoic acid (IOPA) or saline (control)*†

(Mean values with their standard errors for nine birds)

	R+				R−				
	Control		IOPA		Control		IOPA		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P*
Body wt (g)	3223[a]	80	3201[a]	70	3319[a]	90	3275[a]	96	NS
Food intake (g/d)	125[a]	8	117[a]	11	71[b]	4	64[b]	5	***
RFC‡ (g/week)	175[a]	24			−168	37			***
Heat production (J/kg$^{0.75}$ per min)	289[a]	13	230[b]	5	232[b]	11	217[b]	13	***
Physical activity (counts/min)	0·92[a]	0·11	0·94[a]	0·12	0·92[a]	0·10	0·78[a]	0·17	NS
RQ	1·00[a]	0·03	0·93[b]	0·03	1·00[a]	0·04	0·95[b]	0·04	*
Rectal temperature (°)	41·7[a]	0·1	41·8[a]	0·1	41·7[a]	0·2	42·1[a]	0·2	NS
Comb temperature (°)	35·1[a]	0·8	32·8[ab]	1·1	31·2[b]	0·7	31·8[b]	1·1	*

[a,b] Mean values within a row not sharing a common superscript letter were significantly different: $*P < 0{\cdot}05$, $***P < 0{\cdot}001$.

† For details of procedures, see pp. 964–966.

‡ Residual food consumption = difference between observed food consumption (FC) and FC predicted by the regression: FC = a × body weight + b × body-weight gain + c.

Table 5. *Expt 3. Plasma triiodothyronine (T_3) and thyroxine (T_4) concentrations in cockerels divergently selected for high (R−) or low (R+) food efficiency, injected with iopanoic acid (IOPA) or saline (control)*†

(Mean values with their standard errors for nine birds)

	R+				R−				
	Control		IOPA		Control		IOPA		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P*
Plasma T_3 concentration (nmol/l)									
Before injection	$2{\cdot}12^{a}$	0·15	$2{\cdot}04^{a}$	0·13	$1{\cdot}73^{b}$	0·09	$1{\cdot}16^{b}$	0·10	*
After	$2{\cdot}11^{a}$	0·11	$0{\cdot}56^{c}$	0·07	$1{\cdot}70^{b}$	0·13	$0{\cdot}74^{c}$	0·07	*
T_3 decrement	$0{\cdot}01^{c}$	0·11	$1{\cdot}49^{a}$	0·09	$0{\cdot}03^{c}$	0·18	$0{\cdot}87^{b}$	0·07	**
Plasma T_4 concentration (nmol/l)									
Before injection	$23{\cdot}7^{a}$	2·4	$24{\cdot}2^{a}$	2·0	$26{\cdot}2^{a}$	1·1	$26{\cdot}2^{a}$	1·8	NS
After	$25{\cdot}2^{c}$	1·1	$85{\cdot}8^{a}$	5·9	$28{\cdot}6^{c}$	1·8	$72{\cdot}0^{b}$	4·6	*
T_4 increment	$1{\cdot}6^{c}$	2·5	$61{\cdot}6^{a}$	5·8	$2{\cdot}4^{c}$	1·8	$45{\cdot}8^{b}$	4·2	*

[a,b,c] Mean values within a row not sharing a common superscript letter were significantly different: $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$.

† For details of procedures, see pp. 964–966.

DISCUSSION

As reported previously (Géraert *et al.* 1991), R+ birds had a significantly higher food intake than R− birds (range: +49 to +76 %). This could be partly explained by a higher plasma T_3 concentration in the R+ line (May, 1989) and the very low level of fatness observed in this line (Decuypere *et al.* 1987). The excessive energy intake was balanced by increased heat production (+25 %) due to increased DIT (Géraert *et al.* 1991). This enhanced DIT has a regulatory component in the R+ line (Gabarrou *et al.* 1998) which is under β-adrenergic control (Gabarrou & Géraert, 1994*a,b*).

When birds were fed, plasma T_3 concentration and heat production were higher in the R+ than in the R− line, while no difference in plasma T_4 concentration was observed between lines. These results are very similar to those observed previously in overfed human subjects (Danforth *et al.* 1979). When the peripheral conversion of T_4 to T_3 was blocked by iopanoic acid, the differences in T_3 concentration and heat production disappeared, suggesting that the higher heat production of R+ cockerels was related to the higher plasma T_3 concentration. There is ample evidence that thyroid hormone administration increases heat production in mammals (Danforth *et al.* 1979) and birds (Singh *et al.* 1968; Arieli & Berman, 1979; Kittok *et al.* 1982; Hwang-Bo *et al.* 1990). Futher increases in T_3 levels induced by environmental and nutritional changes are associated with an increased resting heat production (Klandorf *et al.* 1981). The present results also suggest a similar relationship between heat production and thyroid hormone levels in fed animals. In the fasting state or after iopanoic acid treatment, however, the large decreases in plasma T_3 were not accompanied by significant reductions in resting metabolic rate (Table 4; Géraert *et al.* 1991; Gabarrou *et al.* 1998). The marked changes in T_4 occurring at the same time may possibly account for part of the difference because T_4 can account for 10–15 % of thyroidal effects (Surks & Oppenheimer, 1977). Alternatively, T_3 may act mainly on regulatory mechanisms producing DIT. Because the difference in

heat production observed between lines has been attributed to DIT (Géraert *et al.* 1991; Gabarrou *et al.* 1998), it is tempting to suggest that this mechanism is partly under the control of plasma T_3 concentration in the R+ line.

To investigate the origin of the higher plasma T_3 concentration observed in the R+ line, peripheral deiodination of T_4 was blocked *in vivo* by a short-term treatment with iopanoic acid in both lines (Decuypere *et al.* 1980). Iopanoic acid caused a greater increase in plasma T_4 and decrease in plasma T_3 in the R+ than in the R− line, suggesting a higher rate of secretion of T_4 and a higher turnover of T_3 in the R+ than in the R− line. These data strongly suggest that there is a higher conversion of T_4 to T_3 in the R+ line. When R+ cockerels were feed-restricted for a long period of time (Expt 1), T_3 concentration decreased slightly, which is consistent with the observation that the level of energy intake influences plasma thyroid hormone levels (Macari *et al.* 1983). The absence of any effect on plasma T_3 when feed-restriction was performed acutely by tube-feeding could be the result of the large amount of energy supplied over a short period of time even in 'restricted' R+ cockerels.

These results suggest that the higher plasma T_3 concentration observed in the R+ birds could be the result of higher deiodinase activity in this line. When hepatic deiodinase activity was measured with addition of exogenous sulfhydryl-groups, no difference was observed between lines. When hepatic deiodinase activity was measured without addition of exogenous sulfhydryl-groups, R+ cockerels exhibited a higher deiodinase activity than the R− cockerels. This result suggests that the maximal capacity of deiodination (i.e. availability of enzyme) is similar in both lines and, thus, is not involved in the line-related difference in plasma T_3 concentration. However, the availability of endogenous sulfhydryl-groups, which markedly affect deiodinase activity (Visser *et al.* 1976), may limit the activity of the enzyme *in vivo*. There are indications from earlier studies that the reduced activity of the enzyme observed in starvation was mainly due to a reduction in non-protein sulfhydryl groups (Balsam & Ingbar, 1979; Harris *et al.* 1979), although other studies have suggested that the reduced co-factor was only of minor importance (Gavin *et al.* 1980). From our data, it appears that the lower hepatic deiodinase activity of the R− line is related to a deficiency of sulfhydryl cofactors *in vivo*, and not to a reduction in the enzyme concentration. The effect was not related to a difference in energy intake because it was also observed in R+ birds pair-fed to the level of R− cockerels. In the present study, long-term food restriction did not markedly affect hepatic deiodinase activity contrary to experiments in pigs (Harrison *et al.* 1996), but the food restriction of adult cockerels was less severe (− 30 % of *ad libitum* food intake) than that of young growing pigs (−50 % of *ad libitum* intake).

Higher plasma T_3 levels and hepatic deiodinase activity in R+ cockerels were associated with higher maximum T_3-binding capacity of hepatic nuclei indicating a higher concentration of T_3 receptors. This could be related both to differences in line and to differences in food intake since previous studies have shown T_3 receptors to be influenced in the long-term by changes in food intake. After 4 weeks of dietary regimens, young pigs provided with a high food intake have a greater number of skeletal muscle nuclear T_3 receptors than their counterparts with a low intake (Dauncey *et al.* 1992). The present findings therefore extend these results to the avian liver. Further, they show that differences in voluntary food intake can also influence tissue concentrations of T_3 receptors. Acute pair-feeding of R+ cockerels to the level of R− birds did not reduce the concentration of T_3 receptors, possibly because the restriction regimen has rather long-term effects. Because short-term changes in T_3 receptors can also take place following a meal (Morovat & Dauncey, 1992) care was taken so that there was the same time lapse between the time of killing and the last meal in all groups of birds.

The higher concentration of T_3 receptors in R+ cockerels may favour the capacity of thyroid hormones to stimulate heat production in the birds. If such a difference in T_3 receptors is maintained in the fasted state, it may help to explain the similar resting heat production despite depressed plasma T_3 levels.

Avian DIT has previously been shown to be mainly under β-adrenergic control in the R+ line (Garrabou & Géraert, 1994*a,b*). T_3 may, therefore, act either directly via an increased number of nuclear receptors and stimulate thermogenesis or by enhancing the sensitivity of tissues to catecholamines (Danforth *et al.* 1979; Bilezikian & Loeb, 1983) by modulating the expression of the genes responsible for either the synthesis or translocation to the plasma membrane of β-adrenergic receptors.

In conclusion, after a meal, R+ cockerels showed a higher heat production associated with higher plasma T_3 concentration than R− birds. This was related to increased hepatic deiodinase activity dependent on the availability of endogenous sulfhydryl groups. Blocking the rise in T_3 with iopanoic acid abolished the rise in heat production. Increased voluntary food intake was associated with increased hepatic T_3 receptor concentration, further potentiating the effects of thyroid hormones. The enhanced DIT of R+ cockerels, balancing their greater energy intake, is therefore controlled by both catecholamines and thyroid hormones. The biochemical mechanisms of avian DIT now need to be determined.

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