Thyroid status, but not insulin status, affects expression of avian uncoupling protein mRNA in chicken

Anne Collin, Mohammed Taouis, Johan Buyse, Ndey B. Ifuta, Veerle M. Darras, Pieter van As, Ramon D. Malheiros, Vera M.B. Moraes, Eddy Decuypere

To cite this version:

HAL Id: hal-02678690
https://hal.inrae.fr/hal-02678690
Submitted on 31 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
You might find this additional information useful...

This article cites 34 articles, 15 of which you can access free at:
http://ajpendo.physiology.org/cgi/content/full/284/4/E771#BIBL

This article has been cited by 4 other HighWire hosted articles:

**Endothermy in birds: underlying molecular mechanisms**
I. Walter and F. Seebacher
[Abstract] [Full Text] [PDF]

**Polymorphisms in Uncoupling Protein, Melanocortin 3 Receptor, Melanocortin 4 Receptor, and Pro-Opiomelanocortin Genes and Association with Production Traits in a Commercial Broiler Line**
P. Sharma, W. Bottje and R. Okimoto
*Poult. Sci.*, October 1, 2008; 87 (10): 2073-2086.
[Abstract] [Full Text] [PDF]

**Effects of Thermal Manipulation During Early and Late Embryogenesis on Thermotolerance and Breast Muscle Characteristics in Broiler Chickens**
[Abstract] [Full Text] [PDF]

**Avian uncoupling protein expressed in yeast mitochondria prevents endogenous free radical damage**
F. Criscuolo, M. d. M. Gonzalez-Barroso, Y. L. Maho, D. Ricquier and F. Bouillaud
[Abstract] [Full Text] [PDF]

Updated information and services including high-resolution figures, can be found at:
http://ajpendo.physiology.org/cgi/content/full/284/4/E771

Additional material and information about *AJP - Endocrinology and Metabolism* can be found at:
http://www.the-aps.org/publications/ajpendo

This information is current as of September 3, 2010.

*AJP - Endocrinology and Metabolism* publishes results of original studies about endocrine and metabolic systems on any level of organization. It is published 12 times a year (monthly) by the American Physiological Society, 9650 Rockville Pike, Bethesda MD 20814-3991. Copyright © 2003 by the American Physiological Society. ISSN: 0193-1849, ESSN: 1522-1555. Visit our website at http://www.the-aps.org/.
Thyroid status, but not insulin status, affects expression of avian uncoupling protein mRNA in chickens

Anne Collin,1,2 Mohammed Taouis,1 Johan Buyse,2 Ndey B. Ifuta,4 Veerle M. Darras,3 Pieter Van As,2 Ramon D. Malheiro,2 Vera M. B. Moraes,2,5 and Eddy Decuyper2

1Station de Recherches Avicoles, Institut National de la Recherche Agronomique, F-37380 Nouzilly, France; 2Laboratory for Physiology of Domestic Animals, Department of Animal Production, Katholieke Universiteit Leuven, B-3001 Leuven and 3Laboratory of Comparative Endocrinology, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium; 4Département de Biologie, Institute Supérieur de la Gombe, Kinshasa/Gombe, République Démocratique du Congo; 5Faculdade de Ciencias Agrárias e Veterinárias, Universidade Estadual Paulista-Jaboticabal, Departamento de Zootecnia, Jaboticabal-Sao Paulo, Brazil 14870–000

Submitted 31 October 2002; accepted in final form 2 December 2002

Collin, Anne, Mohammed Taouis, Johan Buyse, Ndey B. Ifuta, Veerle M. Darras, Pieter Van As, Ramon D. Malheiro, Vera M. B. Moraes, and Eddy Decuyper. Thyroid status, but not insulin status, affects expression of avian uncoupling protein mRNA in chicken. Am J Physiol Endocrinol Metab 284:E771–E777, 2003. First published December 10, 2002; 10.1152/ajpendo.00478.2002.—The aim of this study was to investigate the hormonal regulation of the avian homolog of mammalian uncoupling protein (avUCP) by studying the impact of thyroid hormones and insulin on avUCP mRNA expression in chickens (Gallus gallus). For 3 wk, chicks received either a standard diet (control group), or a standard diet supplemented with triiodothyronine (T3; T3 group) or with the thyroid gland inhibitor methimazole (MMI group). A fourth group received injections of the deiodinase inhibitor iopanoic acid (IOP group). During the 4th wk of age, all animals received two daily injections of either human insulin or saline solution. The results indicate a twofold overexpression of avUCP mRNA in gastrocnemius muscle of T3 birds and a clear downregulation (−74%) in MMI chickens compared with control chickens. Insulin injections had no significant effect on avUCP mRNA expression in chickens. This study describes for the first time induction of avUCP mRNA expression by the thermogenic hormone T3 in chickens and supports a possible involvement of avUCP in avian thermogenesis.

Thyroid status, but not insulin status, affects expression of avian uncoupling protein mRNA in chicken

IN MAMMALS, mitochondrial uncoupling proteins (UCPs) are known to uncouple phosphorylation from oxidation and, hence, to be involved in energy metabolism. Brown fat UCP1 has also been reported to be involved in heat production (for review see Ref. 32). The impact of thyroid hormones on UCP expression is well documented (16, 23, 25), and UCP3 could be one mediator of the thermogenic effect of triiodothyronine (T3) in mammalian skeletal muscle (10). However, the implication of UCP3 in thermogenesis in mammals is controversial (17, 33).

As in mammals, T3 has been reported to have a role in thermoregulatory mechanisms in birds by stimulating heat production (9, 34). However, the involvement of uncoupling mechanisms in such regulation is still unclear. A recent study (31) showed that a UCP homolog called avian (av)UCP is expressed in chicken and duckling muscle. The authors suggest that avUCP is structurally close to mammalian UCP2 and UCP3 but that its function could be nearer to that of UCP1 (expressed exclusively in brown adipose tissue) in mammals. Indeed, the avUCP messenger is overexpressed in cases of cold acclimatization in ducklings and in cockerels from the R+ line presenting a high diet-induced thermogenesis (31). Recent results obtained in our laboratory (6) also suggest that the induction of avUCP mRNA expression in cold-exposed chicks is associated with increased plasma T3 concentrations and heat production. Moreover, avUCP mRNA expression has been shown to be inhibited in chickens early conditioned to heat (37), characterized by low plasma T3 concentrations (39). However, the influence of thyroid hormones on avUCP expression in chickens has not previously been clearly demonstrated.

Insulin is also known to increase mRNA expression of UCP1 in brown adipocytes (38) and UCP2 and UCP3 in rat skeletal muscle in vitro (30). It may thus also be suggested that insulin could regulate avUCP expression in chickens.

The aim of the present study was to investigate hormonal regulation of mRNA expression of avUCP, a gene potentially involved in thermogenesis, by insulin, thyroid hormones, and thyroid hormone metabolism inhibitors in chicken muscle.

MATERIALS AND METHODS

Experimental design. Forty-eight 1-day-old male broiler chicks (Gallus gallus) from a commercial meat-type line (Ross) were purchased from a local hatchery (Avibel, Zoersel, Belgium). Forty-eight 1-day-old male broiler chicks (Gallus gallus) from a commercial meat-type line (Ross) were purchased from a local hatchery (Avibel, Zoersel, Belgium).
HORMONAL REGULATION OF AVIAN UCP EXPRESSION

The conversion of thyroxine (T4) to T3 (9, 29). For the last 5 deiodinase and thyroid hormone metabolism, especially of the thyroid hormones in the thyroid gland (7). A hyperthyroid group (T3 group) received the commercial diet mixed with 1 mg of T3/kg feed (Sigma Chemical). This product is an inhibitor of deiodinase and thyroid hormone metabolism, especially of the conversion of thyroxine (T4) to T3 (5, 29). For the last 5 days of the experiment (week 4), chickens were subjected to their initial treatment, with addition of twice daily intramuscular injections of 0.9% saline solution or 4 U of human insulin/kg body wt (Novo Nordisk, Brussels, Belgium). All groups were fed ad libitum from week 1 to week 4. On day 5 of 4 wk of age (day 26), injections (insulin, saline solution, or iopanoic acid) were planned for birds to be slaughtered 2 h later.

Measurements and blood and tissue sampling. Individual body weights and group feed intakes were measured every week. In week 3 for the IOP group only and for all groups in week 4, body weights were measured every 2 days to calculate the amounts of insulin or IOP to be injected. Amounts were calculated from estimated body weights on the nonweighing days.

On the sampling day (day 26), blood was drawn from a brachial vein with a heparinized syringe and collected in ice-cold tubes to check the effects of the thyroid and insulin treatments on plasma T4 and T3 concentrations and glyce- mia. After the animals were killed by cervical dislocation, the liver and gastrocnemius muscles were excised, frozen in liquid nitrogen, and stored at −80°C for further analysis.

Expression of avUCP. avUCP mRNA expression was determined in gastrocnemius muscles by reverse transcription-polymerase chain reaction (RT-PCR). Total RNAs were isolated using an InstaPure kit (Eurogentec, Angers, France) according to the manufacturer’s recommendations. RNA concentrations were estimated by measuring the absorbance at 260 nm, and purity was assessed by 260/280-nm absorbance by means of a spectrophotometer (Eppendorf, Hamburg, Germany). For RT-PCR analysis, 1 μg of total RNA was reverse transcribed with 200 U of Superscript II RT (Invitrogen, Cergy-Pontoise, France) in the presence of random hexamer primers (0.5 μg/μL, Promega). RT was carried out in the presence of 0.5 mM dNTP mix (Sigma, St-Quentin Fallavier, France) and human placenta RNAguard RNase inhibitor (40 U, Amersham Pharmacia Biotech, Les Ulis, France). The reaction was assayed at 25°C for 15 min and at 42°C for 45 min. PCR was carried out on one-tenth of total RT product in the presence of two sets of primers flanking a 321-bp fragment of avUCP (forward: CTCTACGACTCTGTGAAGCA, reverse: TGTGTCCTTGTAGGCTGTA), and a 148-bp fragment of 18S (forward: CGCGTGATTTATCAGACCA, reverse: ACCCGTGATCCATCGTGA). Annealing and extension were carried out at 58 and 72°C, respectively, over 35 cycles followed by 7 min at 72°C. Negative-control RT-PCR with DNA-free water was included in all experiments. PCR products were electrophoresed on a 1% agarose gel containing 0.075 μL/mL Vistra green (Amersham Pharmacia Biotech). The intensity of RT-PCR bands was determined using a STORM apparatus (Molecular Dynamics).

Plasma analysis. Plasma 3,3',5-triiodothyronine (T3) and T4 concentrations were measured by radioimmunoassay as described by Darras et al. (8). Intra-assay coefficients of variation were 4.5 and 5.4% for T3 and T4, respectively. Antisera to T3 and T4 standards were purchased from Byk-Belga (Brussels, Belgium).

Plasma glucose and triglyceride concentrations were determined by use of commercially available kits from Instrumentation Laboratory (Lexington, KY). Free fatty acid concentrations in plasma were measured using kits from Wako Chemicals (Neuss, Germany) modified for use with the Monarch Chemistry System (Instrumentation Laboratories, Zaventem, Belgium).

Because glycemia was not depressed in the MMI birds receiving the insulin treatment, insulin concentrations were measured by radioimmunoassay on plasma samples from this group treated with insulin and from all groups treated with saline solution, as described by Simon et al. (35) by use of a guinea pig anti-porcine insulin serum (Ab 27–6, gift of G.

Table 1. Experimental design

<table>
<thead>
<tr>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control dietb</td>
<td>Control dietb</td>
<td>Control dietb + saline injection4, n = 6</td>
<td></td>
</tr>
<tr>
<td>n = 12</td>
<td>n = 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control dietb</td>
<td>T3 dietc</td>
<td>Control dietb + insulin injection5, n = 6</td>
<td></td>
</tr>
<tr>
<td>n = 12</td>
<td>n = 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard dietb</td>
<td>MMI dietd</td>
<td>MMI dietd + insulin injection5, n = 6</td>
<td></td>
</tr>
<tr>
<td>n = 48</td>
<td>n = 12</td>
<td>n = 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Killed</td>
<td></td>
</tr>
<tr>
<td>Control dietb</td>
<td>Control dietb + saline injection4, n = 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 12</td>
<td>n = 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control dietb</td>
<td>Control dietb + IOPe</td>
<td>Control dietb + IOPe + saline injection5, n = 6</td>
<td></td>
</tr>
<tr>
<td>n = 12</td>
<td>n = 12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Standard diet was a commercial starter feed (see Buyse et al. (4) for diet composition). 2 Control diet was a commercial grower feed reduced to small pellets by 45 min of mixing. T3 diet was control diet with 1 mg of triiodothyronine (T3)/kg of feed. MMI diet was control diet with 1 g of methimazole/kg feed. 4Saline-treated chicks received 2 daily injections (8:00 AM and 6:00 PM) of 150 μL of 0.9% NaCl solution. 5Insulin-treated chicks received 2 daily injections (8:00 AM and 6:00 PM) of 4 U of human insulin/kg body wt.

AJP-Endocrinol Metab • VOL 284 • APRIL 2003 • www.ajpendo.org
Rosselin, Hôpital Saint-Antoine, Paris, France) and chicken insulin as standard. The intra-assay coefficient of variation was 1.7%.

All measurements were run in the same assay to avoid interassay variation.

Thyroid hormone hepatic concentrations. In normal chickens, liver is the main contributor to plasma T₃ through intracellular conversion of T₄ to T₃ by type I deiodinase. Therefore, the efficiency of the treatment with the deiodinase inhibitor IOP can be evaluated from the effect on intrahepatic T₃ and T₄ concentrations. Thyroid hormones were extracted from livers by homogenization in methanol, extraction in chloroform-methanol (2:1) and two back-extractions in chloroform-methanol-CaCl₂ 0.05% (3:49:48) as described by Gordon et al. (18). The extracts were purified on Bio-Rad AG 1 × 2 resin columns and eluted in 70% acetic acid (24, 27). The addition of labeled [¹²⁵I]T₄ and [¹³¹I]T₃ immediately after homogenization and eluate counting allowed the calculation of an extraction yield, ranging from 50 to 80%, that was used for final calculations. Extracted T₃ and T₄ concentrations were measured by radioimmunoassay (8).

Statistics. Values for individual body weights and body weight gains for the first period (thyroid treatment weeks 2 and 3) and the second period (thyroid and insulin treatment week 4) were analyzed by one-way ANOVA with the thyroid treatment as main effect (Statview, version 5.0; SAS Institute, Cary, NC), followed by a Student-Newman-Keuls test. Due to high heterogeneity of variance between groups, the effects of insulin treatment and thyroid treatment on thyroid hormone concentrations, plasma glucose, triglyceride, and free fatty acid concentrations, and avUCP mRNA expression were analyzed by nonparametric tests, including Kruskal-Wallis tests followed by Mann-Whitney tests. Pearson correlation coefficients were calculated between plasma T₃ concentrations and avUCP mRNA expressions, between plasma and liver thyroid hormone concentrations, and between avUCP mRNA expressions and plasma free fatty acid concentrations.

RESULTS

Plasma and hepatic thyroid hormone concentrations. Plasma thyroid hormone concentrations on day 26, after both thyroid and insulin treatments, are presented in Fig. 1, A and B. As expected from the pharmacological treatments, plasma T₃ concentrations in saline-treated chickens were lower in IOP and MMI groups compared with control birds (1.87 and 0.24 vs. 3.17 pmol/ml; *P < 0.01). As expected, the T₃ treatment induced a threefold increase in plasma T₃ concentrations (*P < 0.05) compared with saline-treated birds.

![Fig. 1.](image1.png)

Fig. 1. A: plasma triiodothyronine (T₃) concentrations (±SE) at 26 days of age in chickens treated with either saline (open bars) or insulin (filled bars) injections. B: plasma thyroxine (T₄) concentrations (±SE) at 26 days of age in chickens treated with either saline (open bars) or insulin (filled bars) injections. Animals received a control diet (control treatment), or a diet containing 0.1% methimazole (MMI treatment) or containing 1 ppm/kg T₃ (T₃ treatment), or a control diet and 2 daily injections of 20 mg/kg body wt of iopanoic acid (IOP treatment). Different letters represent statistically different values between thyroid treatments in saline-treated chickens. *P < 0.05 and **P < 0.01 between saline- and insulin-treated birds within a thyroid treatment.

![Fig. 2.](image2.png)

Fig. 2. A: liver T₃ concentrations (±SE) at 26 days of age in chickens treated with either saline (open bars) or insulin (filled bars) injections. B: liver T₄ concentrations (±SE) at 26 days of age in chickens treated with either saline (open bars) or insulin (filled bars) injections. Animals received control, MMI, T₃, or IOP treatment. Different letters represent statistically different values between thyroid treatments in saline-treated chickens. *P < 0.05 between saline- and insulin-treated birds within a thyroid treatment.
Plasma T₃ concentrations were significantly affected by insulin treatment in the control group (P < 0.01) and the MMI group (P < 0.05): T₃ concentrations were depressed in the insulin-treated control group (2.3 vs. 3.2 pmol/ml), whereas they were enhanced in the insulin-treated MMI group (0.3 vs. 0.2 pmol/ml). Plasma T₃ levels tended to be reduced in the insulin-treated T₃ group compared with the saline-treated T₃ group (−50%, P = 0.10).

In saline-treated chickens, methimazole and T₃ treatments clearly depressed T₄ plasma concentrations compared with control chickens (1.95 and 2.70 vs. 8.17 pmol/ml, respectively; Fig. 1B). Iopanoic acid-treated birds exhibited slightly but nonsignificantly higher T₄ plasma concentrations than control chickens. Plasma T₄ concentrations were not affected by insulin treatment.

Patterns of thyroid hormone concentrations were very similar in the liver and plasma, correlation coefficients being 0.92 for both T₃ and T₄. In saline-treated chickens, hepatic T₃ concentrations were not different in IOP and control birds, whereas concentrations were 91% lower in MMI and 100% higher in T₃ chickens than in control chickens (P < 0.01; Fig. 2A). Insulin treatment depressed hepatic T₃ concentrations only in control and T₃ chickens compared with saline-treated chickens of these groups. The pattern for hepatic T₄ concentrations (Fig. 2B) was the same as the one observed for plasma T₄ concentrations (Fig. 1B).

Plasma glucose, free fatty acid, and triglyceride concentrations. Thyroid treatments did not significantly affect plasma glucose concentrations in saline-treated chickens, except for IOP birds, which exhibited slightly lower glycemia than control birds (2.05 vs. 2.63 g/l; Fig. 3). As expected, insulin treatment decreased glycemia in the control (1.38 vs. 2.63 g/l; P < 0.0001), IOP (1.01 vs. 2.05 g/l, P < 0.05), and especially in the T₃ (0.62 vs. 2.44 g/l, P < 0.0001) groups. However, glycemia was not affected by insulin treatment in MMI chickens.

In saline-treated chickens, thyroid treatment significantly affected plasma triglyceride and free fatty acid concentrations (Table 2): T₃ chickens had lower triglyceride concentrations than IOP and control chickens (P < 0.05) and lower free fatty acid concentrations than control and MMI birds (P < 0.01). In insulin-treated chickens, thyroid treatment did not affect plasma triglyceride concentrations, whereas it affected plasma free fatty acid concentrations (P < 0.05): MMI chickens had lower free fatty acid concentrations than control and T₃ chickens.

Expression of avUCP. avUCP mRNA expression was positively correlated with plasma T₃ concentrations (y = 0.0127x + 0.421, R² = 0.4071, P < 0.001). In saline-treated birds, avUCP mRNA expression was significantly lower in MMI chickens (−74%) and twice as high in T₃ chickens as in control birds (Fig. 4). Treatment with iopanoic acid did not affect avUCP expression (P = 0.37). avUCP mRNA expression was eight times higher in T₃ than in MMI chickens. Insulin treatment did not significantly affect avUCP mRNA expression, irrespective of the thyroid treatment.

avUCP mRNA expressions were not correlated with fatty acid concentrations (coefficient of −0.03, P = 0.86) in the present experiment.

Feed intake and growth. Overall feed intakes during weeks 2 and 3 of the experiment were the highest in the control group and the lowest in the MMI group, these chickens eating 50% less than the control group in week 3. During the 4th wk of the experiment, feed intakes in birds receiving saline solution injections

Table 2. Plasma triglyceride and free fatty acid concentrations

<table>
<thead>
<tr>
<th>Thyroid Treatment*</th>
<th>Control</th>
<th>MMI</th>
<th>T₃</th>
<th>IOP</th>
<th>P</th>
<th>Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma triglyceride concentrations, mg/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline treated</td>
<td>56 ± 5*</td>
<td>85 ± 30ab</td>
<td>34 ± 2b</td>
<td>96 ± 19a</td>
<td>0.049</td>
<td></td>
</tr>
<tr>
<td>Insulin treated</td>
<td>54 ± 6</td>
<td>52 ± 6</td>
<td>41 ± 4</td>
<td>52 ± 8</td>
<td>0.331</td>
<td></td>
</tr>
<tr>
<td>Plasma free fatty acid concentrations, mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline treated</td>
<td>0.51 ± 0.06ab</td>
<td>0.60 ± 0.05a</td>
<td>0.38 ± 0.03b</td>
<td>0.82 ± 0.15a</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Insulin treated</td>
<td>0.59 ± 0.03a</td>
<td>0.33 ± 0.04b</td>
<td>0.63 ± 0.11a</td>
<td>0.59 ± 0.10ab</td>
<td>0.030</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. *Control chicks received control diet; MMI chicks received a diet containing 0.1% methimazole; T₃ chicks received a diet containing 1 ppm T₃/kg; IOP chicks received control diet and 2 daily injections of 20 mg of iopanoic acid/kg body weight. †Concentrations by insulin treatment were analyzed with a Kruskal-Wallis test, the model including the effect of the thyroid treatment (T), followed by Mann-Whitney tests. Different superscript letters within the same row represent statistically different values.
were 30, 81, 112, and 80 g·day⁻¹·animal⁻¹ for the MMI, IOP, control, and T3 groups, respectively. Insulin injections reduced feed intakes in MMI, IOP, control, and T3 groups by 32, 9, 22, and 20%, respectively (Table 3).

Growth was affected by thyroid status. Especially during the 3rd wk of the experiment, body weight gains of MMI, IOP, and T3 chicks were 68, 25, and 29% lower than that of control chicks, respectively, and the same tendency was observed during the 4th wk of the experiment in saline- or insulin-treated chickens. However, body weights did not differ significantly between animals receiving saline solution or insulin on day 26 (data not shown).

**DISCUSSION**

Treatment with T₃ or thyroid inhibitors induced clear changes in thyroid status and dramatic changes in avUCP expression in chickens. As expected, the methimazole treatment strongly depressed both plasma and liver T₄ and T₃ levels as a consequence of the thyroid gland dysfunction (7). The effect of iopanoic acid on T₃ concentrations was less pronounced than that of methimazole, probably because of residual production of T₃ by the thyroid gland (21, 22). In addition, the iopanoic treatment might not have been strong enough to inhibit deiodinases completely. This is suggested by the similar liver T₃ and T₄ concentrations in IOP and control chickens. It is likely that total suppression of the peripheral degradation of T₄ in IOP birds would have significantly increased its plasma levels compared with control birds (9). Nevertheless, T₃ addition in the diet had a clear hyperthyroid effect in T3 chicks, causing a dramatic increase in plasma and liver T₃ concentrations and a decline in plasma and liver T₄ levels. This is probably a consequence of the negative feedback of plasma T₃ on the pituitary release of thyroid-stimulating hormone and, hence, on thyroidal release of T₄ (20).

Thyroid treatment clearly affected avUCP mRNA expression in the same way as plasma T₃ concentrations, in view of the high correlation coefficient between both parameters. avUCP mRNA expression was markedly enhanced by T₃ treatment, whereas it was slightly (but nonsignificantly) depressed by IOP and dramatically depressed by MMI treatment. The poor effect of iopanoic acid on avUCP mRNA expression might be the result of the moderate decline in plasma T₃ concentrations. The results of avUCP gene expression would have been strengthened by measurements of protein expression by Western blot, but assays made with a heterologous antibody were not good, and there is still a need for a specific anti-avUCP antibody. The
increase in avUCP mRNA expression in gastrocnemius muscles of hyperthyroid chickens is consistent with previous results showing the enhancement of UCP3 mRNA expression in skeletal muscle of T₃-stimulated rats (10, 16, 25). However, the implication of UCP3 in thermogenesis in mammals is still being debated (10, 17, 33). Our results are consistent with an involvement of avUCP in thermogenesis in poultry, as suggested by Raimbault et al. (31), where glucagon (a thermogenic hormone) strongly stimulates the expression of this gene. The mRNA expression of avUCP is also markedly increased in cold-acclimatized ducklings and in chickens from the R+ energy-inefficient laying strain (31), both of which present high heat production (1, 11, 14). In contrast, avUCP mRNA expression is reduced in early heat-conditioned chicks, which exhibit lower internal temperatures than nonconditioned chicks (37). It is likely that, as in mammals with UCP1, the enhancement of thermogenesis observed in T₃-treated chickens (9) is partly mediated by increased expression of avUCP. The enhancement of the avUCP gene expression, together with the increases in T₃ β-receptor mRNA expression, β-oxidation, and cytochrome oxidase activities, mitochondrial respiration, and ATP synthesis observed by Mouillet (28) in muscle of ducklings treated with thyroid hormones, could contribute to the thermogenic action of thyroid hormones in birds.

During the last few years, many studies have focused on the roles of mammalian uncoupling proteins UCP2 and UCP3 (12, 17, 33). In particular, Dulloo et al. (12) suggested that UCP3 was more involved in the regulation of lipids as fuel substrate than in thermogenesis, and several authors have suggested that UCPs could facilitate electrophoretic translation of fatty acid RC0O⁻ anions through the mitochondrial internal membrane (15, 36). Indeed, UCP3 mRNA is reported to be upregulated during fasting, which contradicts a role in maintaining thermogenesis through uncoupling mechanisms. Furthermore, nutritional factors [high-fat diet, lipid infusions, fasting (2, 19, 26)] that upregulate the expression of this gene in skeletal muscle also simultaneously enhance plasma free fatty acid levels (17, 40). Evock-Clover et al. (13) recently showed that fasting and the subsequently increased plasma free fatty acid levels were correlated with high mRNA expression of avUCP in chicken. However, in the present study, avUCP mRNA expressions were not correlated with plasma free fatty acid concentrations, which contradicts a role for these plasma metabolites in the mediation of the effects of T₃ or of inhibitor of thyroid metabolism on avUCP mRNA expression in chicken.

In the present experiment, insulin treatment did not significantly affect avUCP mRNA expression either in control or in thyroid-manipulated chickens. This result contrasts with the results observed in mammals, where clear increases in UCP1 mRNA expression were described in brown adipocytes (38) and in UCP2 and UCP3 mRNA expression in rat skeletal muscle in vitro (30). It is possible that the insulin treatment in the present experiment was not strong enough to have clear metabolic effects. However, the results shown in Fig. 3 demonstrate the dramatic effects of insulin treatment on glycemia in control, IOP, and T3 chickens, consistent with the strong effect of insulin in T₃-treated birds already observed by Buyse et al. (3).

The question of the effect of insulin injections remained for insulin-treated MMI chickens that presented glycemia similar to that of saline-treated MMI birds. Yet plasma insulin concentrations measured by radioimmunoassay were more than ten times higher in insulin-treated MMI chickens than in saline-treated MMI birds (665 vs. 43 μU/ml). Thus the absence of effect of insulin in MMI chickens probably results from a resistance to exogenous insulin. Moreover, the present results show that plasma T₃ concentrations tended to be reduced after insulin treatment and that glycemia was the most affected by insulin treatment in the T3 group. This is consistent with recent findings suggesting that glucose is a major driving force for the regulation of peripheral T₃ formation (5). The interactions between thyroid status and insulin signaling must therefore be further investigated in chicken muscle. Our results suggest that, in chickens in vivo, insulin is not a strong regulator of avUCP mRNA expression. However, it is difficult to know whether a single insulin injection or a physiological insulin infusion would also have resulted in unchanged avUCP mRNA expression.

In conclusion, avUCP mRNA expression was strongly regulated by triiodothyronine and the thyroid gland inhibitor methimazole but not by insulin treatment in our conditions. However, further studies are necessary to demonstrate the role of avUCP in uncoupling mitochondria and the relationship with thyroid hormone status.

We thank G. Nackaerts, C. Borgan, A. Respen, L. Noterdaeme, W. van Ham, F. Voets, G. Reyns, and B. Six in Leuven, and S. Crochet and M. Derouet in Tours for their skilled technical assistance. R. D. Malheiros received a postdoctoral fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil.

REFERENCES


