Effects of short term triiodothyronine administration to broiler chickens fed methimazole

R.W. Rosebrough *, B.A. Russell, M.P. Richards

Animal Biosciences and Biotechnology Laboratory, Animal and Natural Resources Institute, United States Department of Agriculture–Agricultural Research Service, Beltsville Agricultural Research Center, Beltsville, MD 20705, USA

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ABSTRACT

The purposes of these experiments were to determine possible relationships among certain indices of lipid metabolism and specific gene expression in chickens (Gallus gallus) fed methimazole (MMI) and the subsequent effects of providing supplemental T3 to relieve the effects of MMI. Male, broiler chickens growing from 14 to 28 days of age were fed diets containing 18% crude protein and either 0 or 1 g MMI/kg of diet. At 28 days, birds received 18% crude protein diets containing either 0 or 1 mg triiodothyronine (T3)/kg. Birds were sampled at 0, 1, 2 & 4 days post relief from MMI or at 0, 3, 6, 9, 24 & 48 h. Measurements taken in the first experiment included in vitro lipogenesis (IVL), malic enzyme (ME), isocitrate dehydrogenase (ICD-NADP), aspartate aminotransferase (AST) enzyme activities and the expression of the genes for ME, fatty acid synthase (FAS) and acetyl coenzyme carboxylase (ACC), ICD and AST. The same enzyme activities and gene expressions were assayed over the intervals mentioned above. In vitro lipogenesis was eliminated due to constraints imposed by sampling times. Gene expression was estimated with real time RT-PCR assays. Dietary MMI decreased IVL and ME at 28 days of age. T3 supplementation for 1 day restored both IVL and ME. Continuing T3 replenishment decreased IVL without affecting ME activity. Although MMI decreased ME gene expression, there was only a transitory relationship between enzyme activity and gene expression when apparent thyroid function was restored with exogenous T3. Metabolic changes in response to feeding T3 occurred within a short period, suggesting that changes in intermediary metabolism preceded morphological changes. Furthermore, the thyroid state of the animal will determine responses to exogenous T3.

1. Introduction

Although the thyroid gland partially controls avian growth, artificial changes in thyroid hormone levels do not always change growth predictably. Leung et al. (1985) reported that dietary triiodothyronine (T3) and thyroxine (T4) decreased body weight and feed efficiency of chickens. Feeding T3 to increase plasma T3 failed to improve growth of dwarf chickens according Leung et al. (1984a). On the other hand, long-term, dietary administration of thyroid hormones in another study decreased both growth and fat deposition, with T3 being more effective than T4 (Decuypere et al., 1987). Other sets of early data also suggested that dietary T3 decreased body fat (Leung et al., 1984b) as well as plasma GH concentrations (Harvey, 1983). It should also be noted that chemical hypothyroidism, caused by either propylthiouracil (PTU) or MMI, also decreased growth (Leung et al., 1984a,b; Chiasson et al., 1979).

What is lacking from these reports is information concerning birds’ recovery from thyroid hormone perturbations (inhibition of T3 production by feeding MMI). Recently, we have proposed and tested the hypothesis that feeding T3 after a 3-week MMI challenge would rapidly restore circulating levels of T3. Methimazole (1-methyl-2-mercaptimidazole), used to induce hypothyroid status in the present experiments, inhibits thyroidal production of thyroid hormones, but does not directly affect extrathyroidal 5′-deiodination of T4.

Malic (ME) enzyme activity was monitored because of its role in providing reducing equivalents (NADPH) for the synthesis of fatty acids. Isocitrate:NADP+ oxidoreductase-[decarboxylating] (ICD) may function as both a residual source for the provision of NADPH and for a coreactant for transamination (α-ketoglutarate). Aspartate aminotransferase (AAT) aids in the removal of excess amine groups formed by feeding high-protein diets by using the previously mentioned α-ketoglutarate as an amine acceptor (Rosebrough et al., 1988).

The purposes of this experiment were to further study the metabolic effects of feeding MMI and repletion of plasma thyroid hormones and to determine if changes in the levels of mRNA for certain lipogenic enzymes relate to changes in metabolic rates associated with
altered thyroid states. We chose to analyze ME, ACC, FAS, ICD and AAT gene expressions because of its central role in providing reducing equivalents, metabolic intermediates and cofactors either supporting or inhibiting de novo lipogenesis. In addition, the rate limiting enzymes of lipogenesis (ACC and FAS) were estimated in vitro by noting lipids formed in vitro when the substrate is acetate (Rosebrough et al., 1988).

2. Materials and methods

Experiment 1. At 14 days of age, male broiler chickens (*Gallus gallus*) were assigned to one of two dietary treatments (Table 1; 18% crude protein + 0 or 1 g MMI/kg diet) for a 14–28-day growth trial. At 28 days, the chickens were given a diet containing 18% crude protein + 0 or 1 mg T3 (T3)/kg to result in two treatment groups (MMI-Control, MMI-T3). The first letter denoted the treatment from 7 to 28 days and the second letter the treatment from 28 to 32 days. The chickens were housed in battery-brooders (4 birds/pen, 900 cm²/pen) in an environmentally controlled room maintained at 20°C–22°C with a 12-h light–dark cycle (0600–1800 h light). Chickens were selected at 0800 to minimize diurnal variation. One chicken was then randomly selected from each of four pen replicates at 28, 29, 30 and 32 days, weighed, and killed by decapitation. The livers were rapidly removed, sectioned into a PBS buffer and washed to remove blood and debris or snap frozen in liquid N2.

Experiment 2. Birds were sampled at 0, 3, 6, 9, 24 & 48 h post relief Experiment 2. Birds were sampled at 0, 3, 6, 9, 24 & 48 h post relief Experiment 2. Birds were sampled at 0, 3, 6, 9, 24 & 48 h post relief Experiment 2. Birds were sampled at 0, 3, 6, 9, 24 & 48 h post relief Experiment 2. Birds were sampled at 0, 3, 6, 9, 24 & 48 h post relief Experiment 2. Birds were sampled at 0, 3, 6, 9, 24 & 48 h post relief Experiment 2. Birds were sampled at 0, 3, 6, 9, 24 & 48 h post relief Experiment 2. Birds were sampled at 0, 3, 6, 9, 24 & 48 h post relief. The livers were rapidly sliced (MacIlwain Tissue Chopper; 0.3 mm). Quad- ruplicate explants were incubated at 37 °C for 2 h in Hanks’ balanced salts containing 10 mM-HEPES and 10 mM-[2-14C] acetate metabolic intermediates and cofactors either supporting or inhibiting de novo lipogenesis. In addition, the rate limiting enzymes of lipogenesis (ACC and FAS) were estimated in vitro by noting lipids formed in vitro when the substrate is acetate (Rosebrough et al., 1988).

2.2. Lipogenic enzyme gene expression

Remaining liver tissues were homogenized (1:10, wt/vol.) in 50 mM-HEPES (pH 7.5)–3.3 mM-[2-14C] acetate (166 MBq/mmol). All incubations were conducted in 3–ml volumes at 37 °C for 2 h under a 95% O2–5% CO2 atmosphere. At the end of the stated incubation periods, the explants were placed in 10 mL of 2:1 chloroform: methanol for 18 h according to Folch et al. (1957). The extracts were evaporated to dryness and dispersed in scintillation fluid. Radioactivity in the extracts was measured by liquid scintillation spectroscopy. In vitro lipogenesis (IVL) was expressed as μmol of acetate incorporated into lipids/g of tissue.

2.1. In vitro techniques

2.1.1. In vitro metabolism—lipogenesis

Livers were sliced (Macilwain Tissue Chopper; 0.3 mm). Quadruplicate explants were incubated at 37 °C for 2 h in Hanks’ balanced salts containing 10 mM-HEPES and 10 mM-sodium [2-14C] acetate (166 MBq/mmol). All incubations were conducted in 3-ml volumes at 37 °C for 2 h under a 95% O2–5% CO2 atmosphere. At the end of the stated incubation periods, the explants were placed in 10 mL of 2:1

<table>
<thead>
<tr>
<th>Ingredient (g/kg)</th>
<th>Soybean meal</th>
<th>Corn meal</th>
<th>Corn oil</th>
<th>Sand</th>
<th>Dicalcium phosphate</th>
<th>Limestone</th>
<th>Sodium Chloride</th>
<th>L-methionineα</th>
<th>Selenium premixb</th>
<th>Mineral premixc</th>
<th>Vitamin premixd</th>
<th>Cellulose</th>
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<td>Sodium Chloride</td>
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<td>L-methionineα</td>
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<td>Vitamin premixd</td>
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<tr>
<td>Cellulose</td>
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<td>Lysine (g/kg)</td>
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Table 1 Composition of the basal diet.

Table 2 Oligonucleotide PCR primers1.

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<th>Gene</th>
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<td>Sense</td>
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<td>ACC</td>
<td>J03541</td>
<td>ACCGTCCTAGATCCTGACCAG</td>
<td>Anti sense</td>
<td>447</td>
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<tr>
<td>FAS</td>
<td>J04485</td>
<td>GCCATCAAAACTTTCATCAGGCC</td>
<td>Anti sense</td>
<td>423</td>
</tr>
<tr>
<td>ME</td>
<td>AF408407</td>
<td>AAATGGATCGTCAGAGGTCG</td>
<td>Sense</td>
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<tr>
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<td>M12105</td>
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<td>201</td>
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<tr>
<td>ICD</td>
<td>XM421965</td>
<td>CCAGTTGAAGGCCAAAGAAG</td>
<td>Anti sense</td>
<td>197</td>
</tr>
</tbody>
</table>

1 ACC = Acetyl-CoA Carboxylase; FAS = Fatty Acid Synthase; ME = Malic Enzyme, AST = Aspartate Aminotransferase, NADP-ICD = NADP-isocitrate dehydrogenase.

2.2. Lipogenic enzyme gene expression

Total RNA was isolated using the Tri-Reagent procedure (Life Technologies, Rockville, MD, USA) and measured spectrophotometrically and qualitatively by agarose gel electrophoresis. RT-PCR reactions (25 μL) consisted of: 1–2 μg total RNA, 1X QuantiTect SYBR Green RT-PCR Master Mix, variable amounts of RNase water, QuantiTect RT mix,

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2.5 mM Mg and 0.5 μM each of each gene specific primer including a set for β-actin, the internal standard. Reverse transcription proceeded for 30 min at 50 °C in the presence of both Omniscript and Serscript. Initially, a 15 min incubation at 95 °C was used to inactivate the RT reaction and to activate the HotStarTaq DNA Polymerase. The following comprised 45–50 PCR cycles: Denaturation for 15 s at 94 °C, followed by annealing for 30 s at 58 °C and extension for 30 s at 72 °C. Fluorescence data were collected in the latter stage by noting SYBR green. Fluorescence data were used to derive the Ct or the PCR cycle to first signification from baseline. The PCR cycle number corresponding to this threshold which is noted as the Ct value may be indicative of gene expression (Bustin, 2000). For example, a small Ct would indicate a high rate of expression because more products would be available to bind the indicator (SYBR green).

Fluorescence data were used to derive the Ct (t) or the PCR cycle to threshold which is noted as the first signification deviation in fluorescence from a base line value. The Ct’s were then transformed to their respective antilogarithmic values. The resultant value was expressed relative to β-actin. β-actin was chosen as a control gene because its Ct was closer to those of the genes studied than other control genes (particularly 18 s RNA). RT-PCR of 18 s RNA gives a Ct (t) far removed from the genes of interest and would mask any changes in rates of gene expression. Lastly, β-actin has not been affected by any treatments that we have imposed in previous experiments. The final values were transformed to their respective reciprocals such that larger values are indicative of greater rates of gene expression. Treatment effects can be determined by noting differences in transformed expression rates.

The real time PCR reactions were validated by several techniques. Reactions were run in the absence of RNA to preclude interference by primer dimerization. Apparent Ct’s in the absence of RNA gave a rate that was several orders of magnitude removed from that noted for gene of interest (presence of RNA and primer of interest). Linearity of reactions rates was noted by using serial dilutions of isolated RNA (0.25, 0.5, 1 & 2 μg of RNA per reaction). Products of the PCR reaction were compared to known DNA standards and DNA melting temperatures were monitored to rule out multiple products. Finally, preliminary reaction samples were sequenced and compare to reported sequences for the respective genes.

2.4 Data analyses used to test the null hypotheses of equality of treatments means

Analyses of variance as described by Remington and Schork (1970) were used to compare the effects of MMI on metabolism at 28 days. The comparisons of interest also involved the effects of restoring thyroid function with either a control diet or supplemental T3 and time following initiation of the latter treatments.

3. Results and discussion

Table 3 describes 1) the effect of dietary MMI on growth of 28 day old broiler chickens and 2) the effect of dietary T3 on growth following relief from MMI feeding. As expected and agreement with our past experiments (Rosebrough et al., 2007), MMI depressed both growth and feed intake (P<0.05) when fed to broiler chickens growing from 14 to 28 days of age. Likewise, supplemental T3 did not increase body weights over those seen in MMI treated birds repleted with just the control diet. In general, based on previously noted, but unpublished data, metabolic parameters were not affected by feed intakes per se. In other words, we have never noted any dose-response effects of feed intake on

<table>
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<tr>
<th>Table 3</th>
<th>Dietary methimazole (MMI) and supplemental T3 on chicken growth.</th>
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<tbody>
<tr>
<td>Feed Consumption a</td>
<td>Body weight c</td>
</tr>
<tr>
<td>Control + MMI</td>
<td>Control + MMI</td>
</tr>
<tr>
<td>850 ± 8.3</td>
<td>724 ± 12.5 + 0.05</td>
</tr>
<tr>
<td>Supplemental T3 given to birds fed MMI from 14–28 days</td>
<td></td>
</tr>
<tr>
<td>Time following T3 initiation</td>
<td>Body weights a</td>
</tr>
<tr>
<td>Days</td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>786 ± 31.0</td>
</tr>
<tr>
<td>2</td>
<td>815 ± 39.5</td>
</tr>
<tr>
<td>4</td>
<td>864 ± 24.4</td>
</tr>
</tbody>
</table>

*Significant difference (P<0.05) at an age period.

a: Chickens were initially offered diets containing 18% crude protein and 0 or 1 g/kg methimazole from 14 to 28 days. Chickens given methimazole were then offered diets containing the same level of crude protein with or without 1 mg T3/kg.

b: Feed consumption from 14 to 28 days.

c: 28-day body weight.

d: Body weights at indicated time periods post initiation of supplemental T3.

25 μmol of [2-14C]acetate incorporated/g of liver into lipids.
metabolism unless intakes were less than 30% of those noted in the ad libitum state.

With the exception of IVL measured only in Experiment 1 (Fig. 1), data are summarized and discussed for both experiments in a common graph for a particular enzyme activity and its corresponding rate of gene expression. It was felt that the two time intervals could be better compared if data were shown together for both experiments on the same graph.

Dietary MMI depressed IVL (Fig. 1, \( P<0.05 \)) at 28 days. Supplementing diets with T3 increased (\( P<0.05 \)) IVL 1 day following initiation of the supplementation. This same supplementation decreased (\( P<0.05 \)) IVL 4 days post initiation of the supplementation compared to the unsupplemented control diet. In contrast, birds previously fed MMI and fed a control diet required at least 4 days to restore IVL. The present study shows that supplemental T3 enhanced lipogenesis in the birds fed MMI, but that enhancement only lasted until restoration of apparent normal levels of plasma T3. At some point, thyroid function in birds fed MMI appears to have been restored by exogenous T3 and further T3 supplementation did not increase IVL. This finding seemed to support previous work by Strait et al. (1989) that also showed the existence of these two mRNA species. The latter work did indicate that the level of ME activity was related to both species. Strait et al. (1989) previously sequenced the 27S species and determined that polyadenylation was responsible for this species size and its sensitivity to T3 administration. They also proposed that a delayed response time in the expression of the 215 fragment was due to a defective polyadenylation site. Thus, message processing may be a further point of regulation of both message stability and translation efficiency. Goodridge’s group (Thurmond and Goodridge, 1998) described T3 response units in the 5'-flanking region of the ME gene. Although this region contained only one major response unit, several minor units were also noted. These minor units, however, were necessary for full gene expression. It is unclear from our data if 1 day restored expression to that noted in the controls at 28 days of age. After 2 days, there was no difference in expression rates resulting from feeding supplemental T3 or just the control diet. In fairness, it should be mentioned that rates for both dietary treatment groups were still greater than that noted in birds fed MMI at 28 days. An examination of short term (particularly from 0 to 24 h) rates of ME gene expression revealed a dramatic increase at 6 h and a further increase at 9 h and a fall in expression 24 h post initiation of T3 supplementation.

Malic enzyme, along with other enzymes controlling lipogenesis, responds to both hypo and hyperthyroid conditions. Thus, ME gene expression and activity are another useful tool in assessing thyroid function in the bird. In contrast to the rat, however, T3 administration did not increase ME activity ten-fold over that noted in the hypothyroid rat (Dozin et al., 1986). Sood et al. (1996) noted that ME mRNA levels in liver followed a pattern of expression similar to that of the ME activity levels. They did show the existence of isoforms of ME mRNA (27S and 21S) with the 27S in much greater quantity. Dozin et al. (1986) indicate that both isoforms coded for ME protein. This finding seemed to support previous work by Strait et al. (1989) that also showed the existence of these two mRNA species. The latter work did indicate that the level of ME activity was related to both species. Strait et al. (1989) previously sequenced the 27S species and determined that polyadenylation was responsible for this species size and its sensitivity to T3 administration. They also proposed that a delayed response time in the expression of the 215 fragment was due to a defective polyadenylation site. Thus, message processing may be a further point of regulation of both message stability and translation efficiency. Goodridge’s group (Thurmond and Goodridge, 1998) described T3 response units in the 5'-flanking region of the ME gene. Although this region contained only one major response unit, several minor units were also noted. These minor units, however, were necessary for full gene expression. It is unclear from our data if
Fig. 3. NADP-linked isocitrate dehydrogenase enzyme activity and gene expression methimazole to deplete thyroid hormones. The experimental protocol is described in Fig. 2. Values for NADP-linked isocitrate dehydrogenase (ICD, EC 1.1.1.42) activity are expressed as μmol of reduced NADP formed per minute under standard assay conditions. Gene expression is noted as the Ct relative to that of the control gene, beta actin. Data are means four chickens at each time point. Bars represent SEM. 

Fig. 4. Aspartate aminotransferase enzyme activity and gene expression in chickens fed methimazole to deplete thyroid hormones. The experimental protocol is described in Fig. 2. Values for aspartate aminotransferase (AAT, EC 2.6.1.1) activity are expressed as μmol of oxidized NAD formed per minute under standard assay conditions. Gene expression is noted as the Ct relative to that of the control gene, beta actin. Data are means four chickens at each time point. Bars represent SEM. 

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occupancy of these response units could play a role in ME gene expression after the initial repletion interval in the present study.

The data in Figs. 3 and 4 should be examined together as we have repeatedly shown that conditions inhibiting fat synthesis stimulate the activities of both ICD and AST (Rosebrough and Steele, 1985; Rosebrough et al., 1988). Furthermore, the above conditions that stimulate lipogenesis also decrease enzyme activities. Fig. 3 shows somewhat inconsistent effects of repletion of ICD activity following the removal of the MMI diet. For example, the control diet appeared to increase activity at 24 h in the second experiment but not in the first experiment. The enzyme activities in the present study indicate that ICD may function in both lipid and protein metabolism by providing a residual capacity for NADPH and a co-reactant for transamination (α-ketoglutarate) and subsequent citrate availability. Providing supplemental T3 to restore thyroid function slightly inhibited gene expression if data were summarized over a 1 to 4 day period. In contrast, both diets had profound effects (P<0.05) on short-term gene expression, especially 6 h post initiation of the treatment. This effect was not sustained any longer that 6 h.

In both experiments, MMI increased (P<0.05) AST activity at 28 days. Providing supplemental T3 increased activity compared to the control diet 1 day following initiation of the treatments. A further examination of effects of feeding either the control or T3 diet revealed highly inconsistent results over shorter sampling intervals. In contrast to its effect on AST activity, MMI decreased (P<0.05) AST expression at 28 days although its effect in the second experiment did not reach significance (Fig. 4). Feeding the T3 diet increased AST gene expression compared to that noted with the MMI diet at 28 days and with that noted by feeding the control diet at 1 and 2 days. An examination of short-term effects showed an effect of T3 supplementation 24 h post initiation of the diet, agreeing with that noted in the first experiment. Refeeding the diet without supplemental T3 resulted in a slight increase (P<0.05) in expression after 1 day and a slight, though insignificant, decrease 2 days posts diet initiation of that diet. Both dietary treatments decreased (P<0.05) expression 4 days post initiation when compared to 28 day values. Short-term sampling (6 h post initiation) revealed a highly significant (P<0.01) decrease in gene expression occurring prior to the previously noted changes in longer-term expression (9–48 h).

Both ICD and AST may function together to affect lipogenesis as competition exists between acetyl-CoA carboxylase and the aconitase-isocitrate dehydrogenase pathway for limited cytoplasmic citrate. Thus, the requirement for α-ketoglutarate as a co-reactant for transamination of excess amino acids depresses citrate levels and the subsequent activation of acetyl CoA carboxylase. Citrate levels appear to control the avian enzyme more than the rat enzyme (Clark et al., 1979; Hillard et al., 1980).

Comparisons of dietary effects in Fig. 5 indicates no statistically significant differences in ACC expression rates in 28-day old birds. In contrast, treating the hypothyroid chickens (dietary MMI) with T3 decreased ACC gene expression at both 1 and 2 days following initiation of the treatment compared to hypothyroid birds given a diet without supplemental T3. An analysis of short-term effects of T3 supplementation showed that gene expression was first stimulated at 6 to 9 h after initiation of supplementation followed by the previously mentioned period inhibition of expression (24–48 h). At 4 days, expression rates were nearly identical for both dietary treatments. Previous work by Hillgartner et al. (1997) showed that T3 enhanced ACC gene expression nearly four fold in the absence of glucose and this effect was magnified by media glucose. The stimulation of ACC activity was a result of an increase in mRNA resulting from changes in transcription rates. The above group also reported a lag time following T3 addition before ACC transcription reached its greatest rate. They inferred that some intermediate was required for the effect of T3 on ACC transcription.

Although our previous findings demonstrate unequivocally that exogenous T3 depresses de novo lipogenesis (Rosebrough and McMurry, 2000), it can also be shown that normal levels of endogenous T3 are required for this effect. Zhang et al. (2001) reported that T3 stimulates a 7-fold increase in transcription of the acetyl-CoA carboxylase-alpha (ACC-alpha) gene in chick embryo hepatocytes suggesting that T3 regulates ACC-alpha transcription by changing the composition of nuclear receptor complexes bound to a ACC-alpha-T3

![Graphs](image)

**Fig. 5.** Acetyl CoA Carboxylase and fatty acid synthase gene expression in chickens fed methimazole to deplete thyroid hormones. The experimental protocol is described in Fig. 2. Gene expression is noted as the Ct relative to that of the control gene, beta actin. Data are means four chickens at each time point. Bars represent SEM. **a** superscript indicates an effect of treatment used to relieve MMI effect (P<0.05). **b** superscript indicates a result of an increase in mRNA resulting from changes in transcription rates. The above group also reported a lag time following T3 addition before ACC transcription reached its greatest rate. They inferred that some intermediate was required for the effect of T3 on ACC transcription.

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response element. At this time, it is important to note that ACC catalyzes a proposed rate-limiting step in de novo lipid synthesis and that T₃ may be necessary for transcription of its gene.

Fig. 5 also summarizes the effects of dietary MMI and T₃ supplementation on FAS gene expression. An examination of both short and longer-term supplementation confirms somewhat paradoxical effects of supplying T₃ to hypothyroid birds. For example, 1 and 2 day values for expression indicate a significant decrease (P < 0.05) in expression accompanying T₃ supplementation. In contrast, there was a significant increase (P < 0.05) in expression 9 h after initiation of the T₃ treatment that occurred prior to the first decrease in expression 24 h post initiation of T₃. In contrast, repleting with the control diet resulted in an apparent increase in expression at 1 and 2 days when compared to that noted in birds fed supplemental T₃.

Swierczynski et al. (1991) found that the addition of triiodothyronine (T₃) to chick-embryo hepatocytes in culture increased accumulations of ME, FAS, ACC and their mRNAs. They proposed that phosphorylation regulated the expression of the above genes whose expression was controlled by T₃. If this is the case, it can be construed that gene expression is controlled by both transcription and post-translational events. Thus it is possible that rapid changed in expression rates as well as certain lag times in the present set of experiments could well be affected by both transcriptional and posttranslational events.

In summary, these data present an interesting contrast to a previous study of ours (Richards et al., 2003) that demonstrated a close correlation between lipogenic gene expression and concomitant enzyme activities. In contrast, the data in this report substantiates previously mentioned study (Richards et al., 2003) was conducted with normal, euthyroid birds undergoing light stimulation to induce egg production. It seems evident that changes in gene expression may control enzyme activity during challenges that radically alter metabolism (fasting–refeeding, high–low protein diets, initial stages of hormone depletion–replenishment) but message stability and enzyme protein turnover may control activity as homeostasis is reached.

References


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