Alterations in nutritional status regulate acetyl-CoA carboxylase expression in avian liver by a transcriptional mechanism

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Feeding previously starved chicks with a high-carbohydrate, low-fat diet stimulates a 9-fold increase in both the rate of synthesis of acetyl-CoA carboxylase (ACC) and the abundance of its mRNA in liver. To define the steps involved in mediating diet-induced changes in the abundance of ACC mRNA, transcriptional activity was measured with the nuclear run-on assay and multiple DNA probes specific to the ACC gene. ACC transcription was low in livers of starved chicks; feeding them with a high-carbohydrate, low-fat diet induced ACC transcription, increasing it 11-fold. An increase in transcription was detectable at 1 h, was maximal at 5 h and remained high for 26 h. Feeding previously starved chicks with a low-carbohydrate, high-fat diet stimulated a smaller increase (4-fold) in the abundance of ACC mRNA and the transcription of ACC than feeding with a high-carbohydrate, low-fat diet. The half-life of ACC mRNA in liver, as estimated from the kinetics of accumulation and decay of ACC mRNA during high-carbohydrate feeding and starvation, was not changed significantly by dietary manipulation. ACC mRNA was expressed at low levels in heart, pectoral muscle, kidney and brain. The abundance of ACC mRNA in these tissues was not affected by nutritional manipulation. These results demonstrate that nutritional control of the abundance of ACC mRNA in the chicken is liver-specific and is mediated primarily by changes in the rate of transcription of the ACC gene.

INTRODUCTION

Acetyl-CoA carboxylase (ACC; EC 6.4.1.2) catalyses the ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA, which is the donor of all except two (ω) of the carbon atoms for the synthesis of long-chain fatty acids. This reaction is considered the rate-limiting step of the fatty acid synthesis pathway [1]. The activity of ACC also modulates the rate of β-oxidation of fatty acids. Malonyl-CoA decreases the transport of long-chain fatty acyl-CoA from the cytoplasm to the mitochondrial matrix by inhibiting carnitine palmitoylacyltransferase I via an allosteric mechanism [2]. The reciprocal effects of ACC activity on fatty acid synthesis and fatty acid oxidation prevent futile cycling of acetyl-CoA to fatty acids and back to acetyl-CoA.

In the liver of avians and mammals, ACC activity is regulated by nutritional status. Short-term changes in enzyme activity are mediated by allosteric and covalent modification mechanisms [1,3–5]. ACC activity is also controlled by long-term alterations in enzyme concentration. For example, the concentration of ACC is low in neonatal chicks, suckling rats and starved chickens and rats; feeding with a high-carbohydrate diet stimulates an 8- to 20-fold increase in the amount of the enzyme [6–12]. The increase in concentration of ACC caused by feeding is dependent on the presence of carbohydrate in the diet; feeding animals with a carbohydrate-free diet does not result in an increase in the concentration of the enzyme.

Alterations in the content of specific nutrients in the diet also affect the concentration of hepatic ACC. Switching rats or chickens from a high-carbohydrate, fat-free diet to one containing fat causes a decrease in concentration of hepatic ACC [13–16]. This effect might be due to an increase in fat intake or a decrease in carbohydrate intake. Using a meal-feeding regimen in which dietary carbohydrate intake was held constant, Clarke and co-workers [13,16] showed that the effects of dietary fat on the concentration of ACC in rats are due mainly to alterations in fat intake. Interestingly, the effect of dietary fat on the concentration of ACC in chickens is due mainly to an alteration in carbohydrate intake [14].

The mechanisms involved in the nutritional control of concentration of ACC have been partly characterized. Changes in the concentration of hepatic ACC caused by starvation/refeeding in rats and chickens and by feeding newly-hatched chicks and suckling rats with a high-carbohydrate diet are due primarily to changes in the rate of synthesis of the enzyme [8,9,12,17,18]. These diet-induced alterations in synthesis of ACC are correlated with comparable changes in the abundance of mRNA species coding for ACC, indicating that regulation occurs at a pretranslational step [10–12,19].

In the present study we assessed the role of two pretranslational processes, transcription and cytoplasmic mRNA stability, in mediating the effects of starvation and refeeding with a high-carbohydrate diet on the levels of hepatic ACC mRNA in the chicken. In addition we investigated the molecular basis by which decreasing the ratio of carbohydrate to fat in the diet inhibits the expression of ACC in liver. Our findings demonstrate that regulation by both of these dietary manipulations is mediated primarily by changes in transcription.

ACC is expressed in many non-hepatic tissues; in some, lipogenesis is not regulated by alterations in nutritional status [6,20–22]. Two isoforms of ACC with molecular masses of 265 and 280 kDa are observed in animal tissues and seem to be derived from separate genes [6,23,24]. We report here that the mRNA encoding the 265 kDa isoform of ACC is expressed at low levels in brain, kidney, heart and pectoral muscle, with the

Abbreviation used: ACC, acetyl-CoA carboxylase.

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latter two tissues exhibiting the lowest levels of expression. Moreover there is no change in the abundance of ACC mRNA in these tissues when starved birds are refed with a high-carbohydrate, low-fat diet.

MATERIALS AND METHODS

Animals

Unincubated embryonated eggs from white leghorn chickens were obtained from Truslow Farms (Chestertown, MD, U.S.A.) and incubated in an electric forced-draught incubator at 37.5 °C and 80 % relative humidity. Newly hatched chicks were placed in brooders with thermostatically controlled heaters and provided with a commercial chick starter diet (Buckeye Starter Grower; analysis by weight: 71 % carbohydrate, 18 % protein, 3 % fat, 5 % crude fibre) and water *ad lib*. A 12 h light/dark cycle (lights on at 06:00h) was maintained throughout the studies. Ten-day-old chicks were subjected to dietary manipulation as described in the results. The composition of the purified diets used in the experiments described in Figures 3 and 4 were as follows. The high-carbohydrate, low-fat diet contained (w/w): 65 % glucose, 25 % isolated soy protein (ICN Biochemicals), 1 % safflower oil, 6 % mineral mix (Briggs chicks salts A; Teklad), 1 % vitamin mix (A.O.A.C.; Teklad), 2 % cellulose and 0.4 % D,L-methionine; the energy content of this diet was 15.36 kJ/g. The low-carbohydrate, high-fat diet contained (w/w): 35 % glucose, 22 % safflower oil, 31 % isolated soy protein, 7.5 % mineral mix, 1.25 % vitamin mix, 2.5 % cellulose and 0.5 % D,L-methionine; the energy content of this diet was 19.21 kJ/g. The levels of protein and other nutrients relative to energy were identical in both of these diets.

Isolation of RNA and quantification of mRNA levels

RNA was extracted from liver, brain, heart, kidney and pectoral muscle by the guanidinium thiocyanate/phenol/chloroform method [25]. Total RNA (15 μg) was separated by size in 0.9 % agarose/0.7 M formaldehyde gels, then transferred to a Nytran membrane (Schleicher & Schuell) with a vacuum blotting apparatus (Pharmacia Biotechnology). The RNA was cross-linked to the membrane by UV irradiation and baked at 80 °C for 30–60 min. RNA blots were hybridized with ^{32}P-labelled DNA probes labelled by random priming [26]. Hybridization and washes were as described [27]. Membranes were subjected to storage phosphor autoradiography. Hybridization signals were quantified with ImageQuant software (Molecular Dynamics).

Nuclear run-on assay of transcription rates

Nuclei were isolated from livers as described by Schibler et al. [28]. Nuclei from two livers were pooled. Approx. 4×10^7 nuclei were stored at -80 °C in 100 μl aliquots containing 50 mM Hepes, pH 7.4, 75 mM NaCl, 0.1 mM EDTA, 5 mM dithiothreitol, 0.125 mM PMSF and 50 % (v/v) glycerol. The transcription reaction *in vitro* was a modification of procedures described by Goldman et al. [29] and Linial et al. [30]. A 100 μl portion of nuclei in storage buffer was mixed with an equal volume of reaction buffer to give the following final concentrations: 25 % (v/v) glycerol, 50 mM Hepes, pH 7.9, 100 mM KCl, 37.5 mM NaCl, 6.5 mM dithiothreitol, 80 μM EDTA, 62.5 μM PMSF, 1 mM ATP, 0.5 mM CTP, 0.5 mM GTP, 150 μCi (1 μM) of [α-^{32}P]UTP, 2 μM unlabelled UTP, 2 mM $MnCl_2$, 35 mM $(NH_4)_2SO_4$, 8.8 mM creatine phosphate, 40 μg/ml creatine phosphokinase and 60 units of RNasin (1 unit inhibits the activity of 5 ng of ribonuclease A by 50 %) (Promega). The reaction was incubated at 26 °C for 20 min and terminated by the addition of 5 mM $MgCl_2$, 5 mM $CaCl_2$ and 50 μg/ml RNase-free DNase I (Gibco/BRL). ^{32}P-labelled transcripts were purified by the method of Linial et al. [30] with NICK columns (Pharmacia Biotechnology) as described in the manufacturer's instructions.

DNA probes were denatured and applied to GeneScreen membranes (Du Pont–New England Nuclear) as described by Salati et al. [31]. The filter-bound DNA was prehybridized for 2 h in 50 % (w/v) formamide, 0.25 M $NaHPO_4$ (1 M $NaHPO_4$ = 134 g of Na_2HPO_4,7H_2O/4 ml of 85 % H_3PO_4 per litre), pH 7.2, 0.25 M NaCl, 7 % (w/v) SDS, 1 mM EDTA, 100 μg/ml denatured salmon sperm DNA and 40 μg/ml yeast tRNA. Hybridization was performed in the same buffer containing ^{32}P-labelled RNA transcripts [(30–50) $\times 10^6$ c.p.m.] for 48 h. The filters were washed sequentially in 2 $\times$ SSC at room temperature (four times for 5 min each); 2 $\times$ SSC plus 10 μg/ml RNase A at 37 °C (once for 20 min); 25 mM $NaHPO_4$, pH 7.2, 1 mM EDTA, 0.1 % (w/v) SDS at 50 °C (twice for 20 min each); and 25 mM $NaHPO_4$, pH 7.2, 1 mM EDTA, 1 % (w/v) SDS at 65 °C (once for 20 min). Hybridization signals were quantified as described above.

DNA probes

Three non-overlapping cDNA fragments of chicken ACC were generated by using cDNA synthesized from chicken liver total RNA and PCR. ACC-1, ACC-2 and ACC-3 correspond to bases 151–2075, 2283–4486 and 5019–6926 respectively of the chicken ACC cDNA sequence reported by Takai et al. [32]. These cDNA fragments are located within the coding region (see Figure 2, lower panel) and encode the 265 kDa isoform of ACC, the only isoform of the enzyme expressed in chicken liver [22]. All three ACC cDNA probes hybridized to two mRNA species of 10 and 11 kb on Northern blots of chicken liver RNA. The mechanism by which the two ACC mRNA species are generated is unknown. Each probe was subcloned into Bluescript (Stratagene) or M13 vectors. The chicken cDNA species for fatty acid synthase, glyceraldehyde-3-phosphate dehydrogenase and β-actin were generously provided by Dr. Gordon G. Hammes (Duke University, Durham, NC, U.S.A.), Dr. Robert Schwartz (Baylor College of Medicine, Houston, TX, U.S.A.) and Dr. Don W. Cleveland (Johns Hopkins University, Baltimore, MD, U.S.A.) respectively. The chicken malic enzyme genomic DNA (ME-4.8-5′) used in nuclear run-on assays has been described [31,33] and was kindly provided by Alan Goodridge (University of Iowa, Iowa City, IA, U.S.A.).

RESULTS

Regulation of the expression of hepatic ACC by starvation and feeding with a high-carbohydrate, low-fat diet

Feeding previously starved chicks with a high-carbohydrate, low-fat diet stimulates an approx. 8–10-fold increase in the abundance of hepatic ACC mRNA [11]. To determine whether this response was mediated by changes in the stability of ACC mRNA, the time course of accumulation and decay of levels of ACC mRNA was measured during feeding and starvation respectively. ACC mRNA levels were low in livers of chicks starved for 24 h; feeding with a high-carbohydrate, low-fat, chick starter diet stimulated a rapid accumulation of ACC mRNA, reaching a new steady-state level of about 9-fold the initial level at 28 h of refeeding (Figure 1A). The half-life of ACC mRNA in fed birds, as estimated from the kinetics of accumulation of ACC mRNA to a new steady state [34], was 3.7 h. When fed birds were starved, levels of ACC mRNA decreased

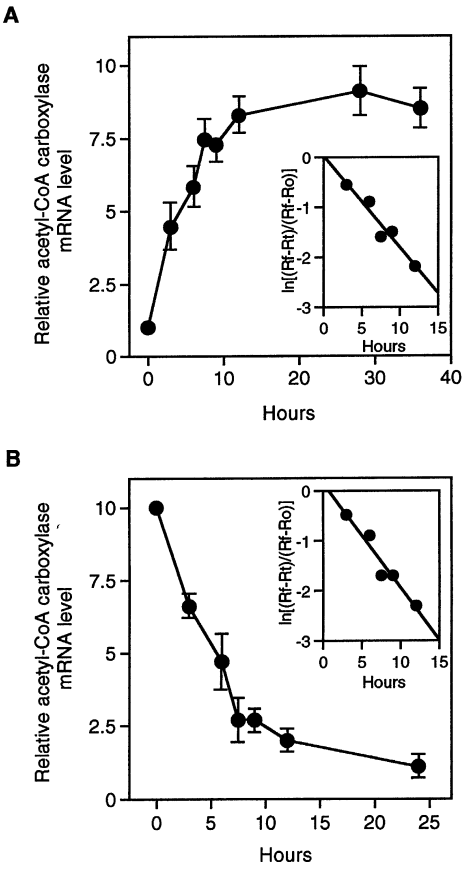

Figure 1 The effects of feeding with a high-carbohydrate diet (a) and starvation (b) on the abundance of ACC mRNA in liver

(**A**) Total RNA was isolated from livers of 10–12-day-old chicks starved for 24 h and then refed with a high-carbohydrate chick starter diet for 0, 3, 6, 7.5, 9, 12, 28 and 36 h. Refeeding began at the beginning of the light period (06:00h). ACC mRNA was measured by Northern analysis as described in the Materials and methods section. Expression of both the 10 and 11 kb species of ACC mRNA was regulated in a similar manner by dietary manipulation. Levels of ACC mRNA at 0 h were set at 1. (**B**) Chicks were fed with a high-carbohydrate diet and then starved for 0, 3, 6, 7.5, 9, 12 and 24 h. Starvation began at 10:00h. Levels of ACC mRNA at 0 h were set at 10. The insets are plots of $\ln[(R_f - R_t)/(R_f - R_0)]$ against time, where R_f is the final RNA concentration, R_0 is the initial RNA concentration and R_t is the RNA concentration at time t during the approach to steady state. The k_d for ACC mRNA during high-carbohydrate feeding (**A**) and starvation (**B**) was estimated from the slopes of the straight lines. Each point is the mean ± SEM for three or four animals. This experiment was repeated once with similar results.

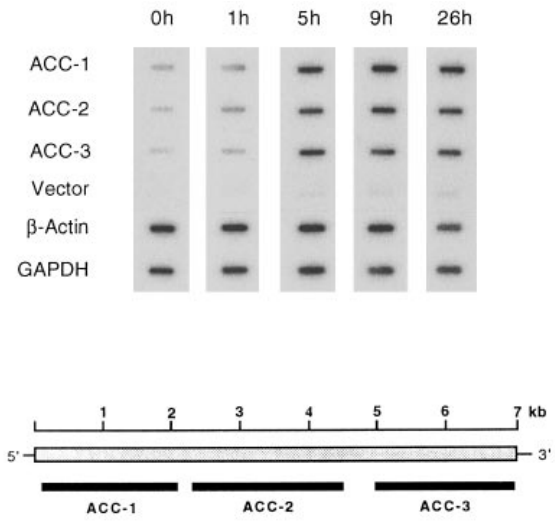

Figure 2 Time course of the effects of refeeding with a high-carbohydrate diet on the transcription of the ACC gene in liver

Nuclei were isolated from livers of 10–12-day-old chicks starved for 24 h and then refed with a high-carbohydrate chick starter diet for 0, 1, 5, 9 and 26 h. Feeding began at the beginning of the light period (06:00h). Livers from two birds were pooled for each time point. Nuclear run-on assays were performed as described in the Materials and methods section. Bluescript vector DNA was a control for non-specific hybridization. The lower panel shows the ACC cDNA fragments that were used as probes in the nuclear run-on assay. The stippled rectangle represents the coding sequence of the ACC cDNA. This experiment was conducted a total of three times with similar results. Abbreviation: GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

rapidly, reaching a new steady state at about 24 h (Figure 1B). The half-life of ACC mRNA in starved birds was estimated to be 3.3 h. These results indicate that changes in nutritional status have little or no effect on the stability of ACC mRNA.

To determine whether alterations in transcription were responsible for diet-induced changes in the abundance of ACC mRNA, nuclear run-on assays were performed with nuclei isolated from livers of starved chicks and chicks refed with the high-carbohydrate, low-fat, chick starter diet. Multiple DNA probes were employed in the assays to distinguish regulation at initiation of transcription from regulation at elongation or termination of transcription. ACC-1, ACC-2 and ACC-3 are non-overlapping ACC cDNA fragments that represent the 5′, middle and 3′ portions respectively of the coding region of chicken ACC (Figure 2). Each probe was characterized to ensure the validity of the results of the nuclear run-on assay. Briefly, on the basis of Southern analysis of genomic DNA, ACC-1, ACC-2 and ACC-3 are unique and do not contain repetitive sequences (results not shown). Nuclear transcripts generated during elongation *in vitro* hybridized only to the coding strand of each ACC probe, indicating that transcription was strand-specific. In chicks starved for 24 h, the transcription rate of the ACC gene as detected by ACC-1 was low; feeding with the high-carbohydrate, low-fat, chick starter diet stimulated a 2.5 ± 0.2 (S.E.M.)-fold increase in transcription at 1 h of refeeding (Figure 2). Transcription of ACC increased to the maximal extent at 5 h (11.2 ± 1.5-fold) and remained high at 9 h (12.8 ± 0.6-fold) and 26 h (10.8 ± 0.8-fold) of refeeding. Similar results were obtained with ACC-2 and ACC-3. Starvation and feeding had no significant effect on the transcription of the genes for β-actin and glyceraldehyde-3-phosphate dehydrogenase, indicating that the effects of this dietary manipulation on transcription are selective for ACC.

Regulation of hepatic ACC expression by the level of carbohydrate in the diet

In addition to starvation and refeeding, alterations in the content of specific nutrients in the diet control the expression of ACC. The activity of hepatic ACC is higher in chickens fed with a high-carbohydrate, low-fat diet than in chickens fed with a low-carbohydrate, high-fat diet [14,15]. This effect is mainly due to an alteration in carbohydrate intake [14]. To investigate the molecular basis for the regulation of expression of ACC by the level of carbohydrate in the diet, the abundance of ACC mRNA was measured in livers of previously starved chicks fed with a high-carbohydrate, low-fat, purified diet (65% glucose, 1% safflower oil) or a low-carbohydrate, high-fat purified diet (35% glucose, 22% safflower oil) for 28 h. The ratio of protein to energy in

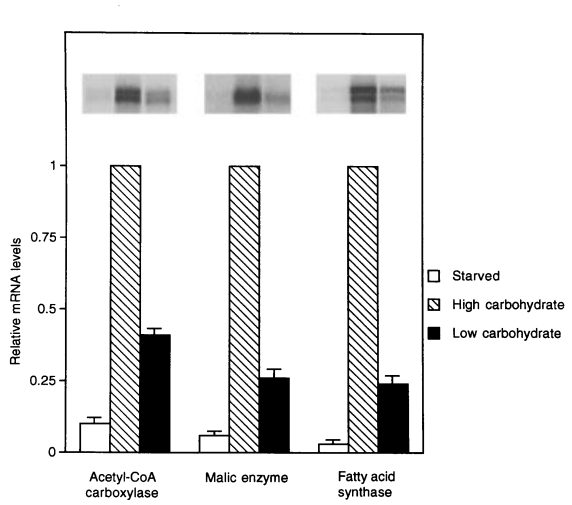

Figure 3 Effects of altering the carbohydrate content of the diet on the abundance of the mRNA species for ACC, malic enzyme and fatty acid synthase in liver

Total RNA was isolated from livers of chicks starved for 24 h and then refed with a high-carbohydrate, low-fat diet or a low-carbohydrate, high-fat diet for 0 and 28 h. The composition of the diets is described in the Materials and methods section. The abundances of the mRNA species for ACC, malic enzyme and fatty acid synthase were measured by Northern analysis. The bars represent the means ± S.E.M. for mRNA levels from five animals. In birds fed with the high-carbohydrate, low-fat diet, mRNA abundance was set at 1. The insets show representative mRNA bands for each dietary condition.

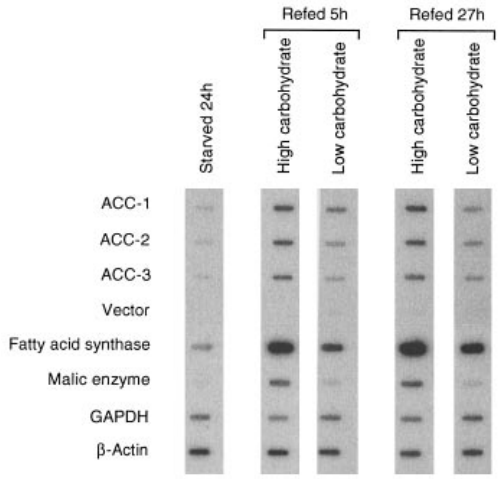

Figure 4 Effects of altering the carbohydrate content of the diet on the transcription of ACC, fatty acid synthase and malic enzyme in liver

The experimental design was similar to that described in Figure 3 except that chicks were fed with the diets for 0, 5 and 27 h. Nuclei were isolated and nuclear run-on assays performed as described in the Materials and methods section. Livers from two animals were pooled at each time point. This experiment was repeated twice with similar results.

these two diets was identical. The abundance of ACC mRNA was 2.5-fold higher in chicks fed with the high-carbohydrate, low-fat diet than in chicks fed with the low-carbohydrate, high-fat diet (Figure 3). Levels of ACC mRNA in chicks fed with the low-carbohydrate, high-fat diet were increased 4-fold relative to those of starved chicks. To determine whether the effects of dietary carbohydrate on the abundance of ACC mRNA were mediated by changes in the transcription rate of the ACC gene, nuclear run-on assays were performed. After 5 h of refeeding, transcription of ACC was 2.4 ± 0.3-fold higher in chicks fed with the high-carbohydrate, low-fat diet than in chicks fed with the low-carbohydrate, high-fat diet. Rates of transcription of ACC in chicks fed with the low-carbohydrate, high-fat diet were increased by 3.5 ± 0.4-fold relative to those of starved chicks (Figure 4). Similar differences in the transcription of ACC between high-carbohydrate-fed chicks and low-carbohydrate-fed chicks were observed after 27 h of refeeding. Alterations in the carbohydrate content of the diet had no significant effect on the abundance of mRNA (results not shown) and the transcription rates (Figure 4) of β-actin and glyceraldehyde-3-phosphate dehydrogenase.

As with ACC, the activities of malic enzyme and fatty acid synthase are higher in chicks fed with a high-carbohydrate, low-fat diet than in chicks fed with a low-carbohydrate, high-fat diet [14,15,35]. The co-ordinated regulation of these enzymes suggests that common regulatory mechanisms are involved at the molecular level. To investigate this possibility, the abundances and transcription rates of mRNA species for malic enzyme and fatty acid synthase were also measured in livers of chicks fed with purified diets containing different amounts of carbohydrate. The abundances of mRNA species for both malic enzyme and fatty acid synthase were 4-fold higher in chicks fed with the high-carbohydrate, low-fat diet than in chicks fed with the low-carbohydrate, high-fat diet (Figure 3). Levels of malic enzyme and fatty acid synthase mRNA species in chicks fed with the low-carbohydrate, high-fat diet were increased by 4.2-and 7.6-fold respectively relative to those in starved chicks. These diet-induced changes in the abundances of mRNA species for malic enzyme and fatty acid synthase were accompanied by similar alterations in the transcription rates of these genes (Figure 4). After 5 h of refeeding, transcription of malic enzyme and fatty acid synthase was increased by 4.5 ± 0.6-fold and 3.6 ± 0.8-fold respectively in chicks fed with the high-carbohydrate, low-fat diet relative to chicks fed with the low-carbohydrate, high-fat diet. The transcription rates of malic enzyme and fatty acid synthase in chicks fed with the low-carbohydrate, high-fat diet were increased by 4.0 ± 0.6-fold and 7.9 ± 1.4-fold respectively relative to those of starved chicks. Similar differences in the transcription of malic enzyme and fatty acid synthase between high-carbohydrate-fed chicks and low-carbohydrate-fed chicks were observed after 27 h of refeeding.

ACC expression in non-hepatic tissues

ACC activity is present in many non-hepatic tissues [20–23]. We were interested in analysing the tissue-specific expression and regulation of ACC mRNA in the chicken. The abundance of the mRNA encoding the 265 kDa isoform of ACC was measured in heart, pectoral muscle, kidney and brain of starved chicks and chicks refed with a high-carbohydrate, low-fat diet (Figure 5). Two species of ACC mRNA of about 10 and 11 kb were detected in all tissues. In starved chicks, the levels of ACC mRNA in heart, pectoral muscle, kidney and brain were decreased relative to those in liver. Heart and pectoral muscle exhibited the lowest expression of ACC mRNA. In contrast with liver, the abundance of ACC mRNA in non-hepatic tissues was not affected by nutritional manipulation.

DISCUSSION

The results of the present investigation provide several lines of evidence indicating that transcription initiation is the primary step involved in the nutritional regulation of the abundance of

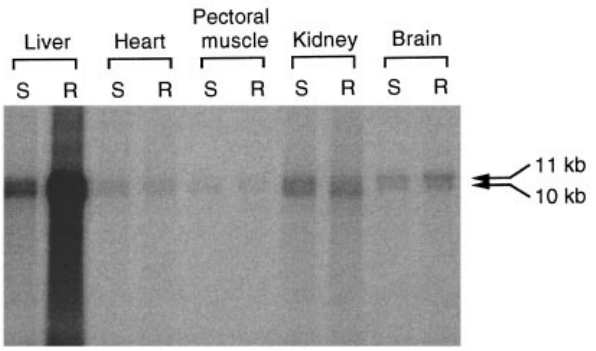

Figure 5 Tissue-specific expression and regulation of ACC mRNA

Total RNA was isolated from liver, heart, pectoral muscle, kidney and brain of 12–14-day-old chicks starved for 24 h and then refed with a high-carbohydrate chick starter diet for 0 h (S) or 28 h (R). Each RNA preparation was isolated from the pooled tissues of two animals. Northern analysis was performed as described in the Materials and methods section. The positions and sizes of the mRNA species for ACC are indicated by the arrows. Similar results were observed in three independent experiments.

ACC mRNA in chicken liver. (1) Three cDNA fragments corresponding to the 5′, middle and 3′ portions of the coding region of chicken ACC mRNA were used as probes in the nuclear run-on transcription assay. An 11-fold increase in transcription of ACC was detected by each probe when previously starved chicks were fed with a high-carbohydrate, low-fat diet. The stimulation of transcription of ACC was dependent on the level of carbohydrate in the diet. Transcription of ACC was about 2.4-fold higher in chicks fed with a high-carbohydrate, low-fat diet than in chicks fed with a low-carbohydrate, high-fat diet. The magnitudes of these diet-derived changes in transcription of ACC were sufficient to account for alterations in steady-state levels of ACC mRNA. (2) The stimulation of transcription of ACC in starved chicks refed with a high-carbohydrate, low-fat diet preceded the accumulation of ACC mRNA. An increased rate of transcription of ACC was detectable at 1 h and was maximal at 5 h of refeeding. The increase in levels of ACC mRNA was maximal at about 28 h of refeeding. (3) Starvation and refeeding with a high-carbohydrate, low-fat diet had no effect on the stability of ACC mRNA as estimated from the kinetics of accumulation and decay of ACC mRNA. This finding is consistent with the results of nuclear run-on measurements indicating that post-transcriptional processes play little or no role in the dietary control of levels of ACC mRNA.

The role of transcription in mediating diet-induced changes in the expression of ACC has also been investigated in the rat. In livers of starved rats, the abundance of ACC mRNA is low; it increases 9–12-fold after refeeding with a high-carbohydrate, low-fat diet [10,19]. The transcription rate of the ACC gene increases only by 2.5-fold during refeeding [19]. Thus diet-induced changes in the abundance of ACC mRNA in rat liver seem to be mediated primarily by a post-transcriptional mechanism. This observation contrasts with our results in the chicken showing that nutritional control of the expression of ACC is primarily transcriptional. It is unclear whether this discrepancy is due to differences in classes of experimental animals.

The rat ACC gene is transcribed and processed into at least five different mRNA species that contain the same coding sequence but different 5′ untranslated regions [36,37]. The use of two different promoters and alternative processing is responsible for the generation of the different transcripts. The two promoters of the rat ACC gene are separated by approx. 12.5 kb. The products of these promoters are expressed in a tissue-specific manner. In rat liver, ACC mRNA species are generated from both promoters; the levels of these mRNA species are regulated by starvation and refeeding with a high-carbohydrate, fat-free diet [38]. ACC mRNA species in rat adipose tissue are the products of only the more upstream promoter (PI); the levels of these mRNA species are also regulated by dietary manipulation [39]. ACC mRNA species in lactating mammary tissue are the products of only the more downstream promoter (PII). In the chicken, less is known about the nature of the mRNA species generated from the ACC gene. Recently, 5′ flanking DNA of the chicken ACC gene has been cloned and a promoter has been identified [40]. This promoter generates an mRNA in chicken liver that is similar in structure to the major ACC mRNA expressed from PII in rat liver. It is currently unclear whether additional ACC mRNA species are generated by a second promoter in the chicken ACC gene. If ACC mRNA species in chicken liver are the products of two promoters, additional studies will be needed to determine whether the activities of one or both of these promoters are regulated by dietary manipulation.

Alterations in nutritional status regulate the transcription of other lipogenic enzymes in chicken liver. Starvation or feeding with a low-carbohydrate, high-fat diet causes comparable decreases in the mRNA abundance and transcription rates of fatty acid synthase and malic enzyme; feeding with a high-carbohydrate, low-fat diet reverses these effects (Figures 3 and 4) [33,41]. Thus dietary control of the transcription of fatty acid synthase and malic enzyme is co-ordinate with that of ACC. This observation is consistent with the hypothesis that common mechanisms are involved in the nutritional regulation of these enzymes in the chicken [42]. Further testing of this hypothesis will require the identification and characterization of the *cis*-acting sequences and *trans*-acting factors that mediate the effects of diet on the transcription of the avian genes for ACC, fatty acid synthase and malic enzyme.

In previous reports the concentration of the 265 kDa isoform of ACC has been measured in various tissues with immunological methods [6,11,20–22]. In general, the results of these studies are consistent with those of the present study, demonstrating that the expression and nutritional regulation of the mRNA encoding the 265 kDa isoform is tissue-specific. The identification and characterization of the factors involved in the tissue-specific control of the avian ACC gene should be facilitated by the availability of hepatic and non-hepatic cell culture systems that mimic this regulation *in vitro* [43].

In conclusion, the nutritional regulation of ACC mRNA levels in the chicken is liver-specific. Diet-induced changes in ACC mRNA abundance are primarily due to alterations in the transcriptional activity of the ACC gene. The identification of the humoral factors that signal changes in dietary status to the liver and the elucidation of the biochemical mechanisms through which these signalling molecules bring about changes in ACC transcription are areas of active investigation in our laboratory.

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