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Adiponectin Gene Is Expressed in Multiple Tissues in the Chicken: Food Deprivation Influences Adiponectin Messenger Ribonucleic Acid Expression

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Adiponectin is a cytokine hormone originally found to be secreted exclusively by white adipose tissue. However, recent evidences suggest that adiponectin is also produced in brown adipose tissue and skeletal muscle. The present study investigated the expression of adiponectin mRNA in various tissues in the chicken. We also studied the effect of food deprivation on adiponectin gene expression in adipose tissue, liver, anterior pituitary gland, and diencephalon in the chicken. The open reading frame of chicken adiponectin cDNA consists of 735 nucleotides that were 65–68% homologous to various mammalian adiponectin cDNAs. The deduced amino acid sequence of chicken adiponectin contains 22 glycine-X-Y repeats (in which X and Y represent any amino acid) at the N-terminal end as found in the mammalian adiponectin. By RT-PCR and Northern analysis, we detected chicken adiponectin mRNA transcript in adipose tissue, liver, anterior pituitary gland, diencephalon, skeletal muscle, liver, kidney, ovary, and spleen but not in blood. Adiponectin mRNA expression in various tissues was quantitated using real-time quantitative PCR and found to be the highest in adipose tissue, followed by liver, anterior pituitary, diencephalon, kidney, and skeletal muscle. We also found that adiponectin mRNA quantity was significantly decreased after a 48-h food deprivation in adipose tissue, liver, and anterior pituitary gland but not in diencephalon. Our results provide novel evidence that, unlike mammals, adiponectin gene is expressed in several tissues in the chicken and that its expression is influenced by food deprivation. (*Endocrinology* 146: 4250–4256, 2005)

ADIPONECTIN (also called Acrp30, apM1, GBP28, or adipoQ) is a 30-kDa adipocytokine hormone exclusively secreted from the adipose tissue in several mammalian species (1–4). It is a 244-amino-acid protein belonging to a family of proteins that contain sequences homologous to the C1q globular domain (5, 6). Adiponectin consists of a N-terminal collagenous domain and C-terminal globular domain (7, 8). It circulates as a trimer, hexamer, heavy molecular weight forms, and also as small proteolytic cleavage products in mouse and human plasma (9, 10). Adiponectin exerts its action by binding to two specific receptors, adipoR1 and adipoR2 (11). AdipoR1 is expressed ubiquitously, with the most abundant expression occurring in the mouse skeletal muscle, whereas adipoR2 is predominantly expressed in the mouse liver (11). Expression of adipoR1 and adipoR2 has been reported in human and rat pancreatic β-cells (12) as well.

Adiponectin has been found to have a profound effect on glucose utilization, insulin sensitivity, lipid synthesis, and energy homeostasis in several mammalian species. Deletion of the adiponectin gene induces insulin resistance in mice fed with high-fat/high-sugar diet (13). Adiponectin treatment or overexpression of the adiponectin gene protects overfed rats from gaining weight and from developing cardiovascular diseases by suppressing glucose production and enhancing lipid oxidation (13, 14). Overfeeding and obesity decreases blood adiponectin concentrations, whereas calorie restriction results in increased adiponectin levels and insulin sensitization in rats (15). Overexpression of adiponectin also significantly decreases free fatty acids and triglycerides in genetically obese mice (16). In addition, adiponectin acts in the brain to bring about increased energy expenditure throughout the body, resulting in significant body weight reduction in mice (17). Adiponectin has been found to antagonize the incorporation of glucose carbon into lipid in the cultured porcine adipocytes (18). Adiponectin stimulates glucose utilization and fatty-acid oxidation by phosphorylating and activating 5′-AMP-activated protein kinase (14). Such activation also results in increased muscle fatty acid oxidation through inhibition of acetyl-Coenzyme A carboxylase and activation of malonyl-Coenzyme A decarboxylase (19). Furthermore, transgenic overexpression of adiponectin in rats significantly decreased gluconeogenesis and lipogenesis by inhibiting gene expressions of phosphoenolpyruvate carboxykinase and sterol regulatory element-binding protein 1c in the liver (20).

Whereas adiponectin has been well characterized in mammalian species, nonmammalian adiponectin has not been described in the past. Adiponectin is likely to play a dominant role in carbohydrate and lipid metabolism in avian species because chickens maintain a very high blood glucose concentration (21). In addition, a majority of the lipids in the chicken are synthesized in the liver but not in the adipose tissue as in mammals (22). We report characterization of chicken adiponectin cDNA that was cloned from a pituitary cDNA library. We provide novel evidence that, unlike in

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Abbreviations: DNAse-I, Deoxyribonuclease I; No-RT control, RT reactions where reverse transcriptase was omitted.

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mammals, adiponectin gene is expressed in the chicken liver, diencephalon, anterior pituitary gland, kidney, ovary, spleen, and skeletal muscle besides adipose tissue. We also tested the hypothesis that fasting would affect adiponectin mRNA expression in adipose tissue, liver, pituitary gland, and diencephalon in the chicken.

Materials and Methods

Animals

Sexually mature egg-laying 35-wk-old hens (Hyline W36 strain) housed in cages were provided water and standard layer feed *ad libitum* unless otherwise indicated. All animal procedures were performed in accordance with the Institutional Animal Care and Use Committee approved protocol. Chickens were fasted for 48 h to determine the effect of fasting on adiponectin gene expression in various tissues. Water was provided *ad libitum* during fasting.

Cloning of chicken adiponectin cDNA

Adiponectin cDNA was cloned from a chicken pituitary cDNA library developed in our laboratory using anterior pituitary glands collected from 85-wk-old recycled egg-laying chickens (Hyline W36 strain). For creating the pituitary cDNA library, total RNA from the pituitary gland was extracted using RNeasy kit (Qiagen, Valencia, CA). Both quality and quantity of total RNA was determined using a spectrophotometer (Amersham Biosciences, Newark, NJ). After deoxyribonuclease I (DNAse-I) treatment, total RNA was reverse transcribed to obtain the first-strand cDNA using Powerscript reverse transcriptase using Smart IV oligonucleotide and CDS III/3′ PCR primer set (Creator Smart cDNA Library Construction kit; Clontech, Palo Alto, CA). The resultant single-stranded cDNA was amplified using 5′ PCR primer and CDS III/3′ primer (Clontech) in a PTC-200 DNA engine (MJ Research, Reno, NV) using initial denaturation at 95 C for 1 min, followed by 35 cycles of 95 C for 15 sec and 68 C for 6 min. After proteinase K inactivation of DNA polymerase activity, the cDNAs were digested with *Sfi*I, size fractionated using CHROMA SPIN-400 column and cloned into pDNR-LIB (Clontech) plasmid, and transformed into Electromax competent cells (Invitrogen, Carlsbad, CA). The transformed cells were grown in LB agar media containing chloramphenicol. The resulting library was tittered and amplified. Using the amplified library, plasmids were purified using a Maxiprep kit (Marligen Biosciences, Ijamsville, MD). A PCR-based library screening was done to detect and amplify adiponectin cDNA using primers designed from a chicken adiponectin cDNA sequence (GenBank accession no. AY523637). A 735-bp PCR product was amplified using 300 nM of the forward and reverse primers (forward, ATGAGGGGCTCAGTAGGCTTCCTCCTTT; reverse, CTAACGGTCATCTGTGTCTGGGTACAGGAGGAA), 300 nM dNTP mixture (Invitrogen), and 2.5 U of Deep Vent$_R$ polymerase (New England Biolabs, Beverly, MA). The mixture was subjected to PCR using a thermocycle of 95 C for 2 min, 35 cycles of 95 C for 30 sec, 55 C for 10 sec, 68 C for 1 min, with a final extension at 68 C for 5 min. The 735-bp PCR product was gel purified and subcloned into pGEM-T Easy vector (Promega, Austin, TX), and both sense and antisense strands were sequenced (Davis Sequencing, Davis, CA) using Sp6 and T7 primers.

RT-PCR

Adipose tissue, diencephalon, blood, anterior pituitary gland, liver, skeletal muscle, spleen, ovary, and kidney were harvested from sexually mature 35-wk-old female chickens. Total RNA from each tissue was extracted using Trizol (Invitrogen) and/or by using RNeasy kit (Qiagen). After DNAse-I (Qiagen) treatment, first-strand cDNA was synthesized by reverse transcribing 1 μg of total RNA using d(T)$_{30}$A/G/CA/G/C/T primer, and 2U Powerscript reverse transcriptase (Clontech). Approximately 50 ng of single-stranded cDNA was used as template to amplify a 350-bp product [nucleotides 250–599 of chicken adiponectin cDNA (Fig. 1)] using the following primers: forward, ACAGGTGCAGAAGGACCGAGAGGATT; reverse, AAGACAGAGCCGCTTGCTTGGTCAAC. A touch-down PCR was performed using the following program: 94 C for 1 min, 30 cycles of 94 C for 5 sec, and 72–68 C for 3 min. Annealing and primer extension were done at 72, 70, and 68 C during 1–5, 6–10, and 11–30 cycles, respectively. The PCR products were subjected to agarose gel electrophoresis and ethidium bromide staining for visualization. For negative control, RT reactions using 1 μg of total RNA from each tissue with no reverse transcriptase (No-RT control) were used as template in place of single-stranded cDNA.

Northern hybridization analysis

Total RNA was extracted from the diencephalon, blood, pituitary gland, liver, skeletal muscle, and adipose tissue using Trizol (Invitrogen) and/or by using RNeasy kit (Qiagen) and treated with DNAse-I. Total RNA (1 μg) from each tissue was electrophoresed, transferred to nylon membrane (Amersham Biosciences), and UV cross-linked. A 350-bp chicken adiponectin partial cDNA was amplified by PCR (corresponding to nucleotides 250–599 in Fig. 1). T7 polymerase promoter sequences were added to the antisense strand of this product by PCR. A digoxigenin-labeled riboprobe was prepared by *in vitro* transcription using T7 polymerase and used in Northern hybridization. The membranes were hybridized with the riboprobe overnight at 68 C. After high-stringency washes at 68 C, the membranes were incubated with anti-digoxigenin antibody conjugated to alkaline phosphatase (Roche Biochemicals, Indianapolis, IN), and the hybridization signal was detected using ECF chemifluorescent substrate (Amersham Biosciences). Hybridization signal was detected using Storm 860 imager (Molecular Dynamics, Piscataway, NJ).

Adiponectin mRNA quantitation in various tissues by real-time quantitative PCR

Total RNA was extracted from adipose tissue, diencephalon, pituitary gland, skeletal muscle, liver, and kidney as described above. Total RNA (1 μg) was reverse transcribed using d(T)$_{30}$A/G/CA/G/C/T, 2U Powerscript reverse transcriptase (Clontech) in a 20-μl reaction. Both adiponectin mRNA and chicken β-actin mRNA were quantitated using 2 μl of the RT reaction (equivalent of 100 ng of single-stranded cDNA) as template in the real-time quantitative PCR. An 86-bp product for chicken adiponectin corresponding to nucleotides 204–289 (Fig. 1) was amplified using the following primers: forward, GCCAGGTCTACAAGGTGTCA; reverse, CCATGTGTCCTGGAAATCCT. Similarly, a 123-bp product of chicken β-actin corresponding to nucleotides 1026–1148 (GenBank accession no. L08165) was amplified using the following primer set: forward, CTGGCACCTAGCACAATGAA; reverse, CTGCTTGCTGATCCACATCT. The real-time quantitative PCR consisted of 1× Platinum SYBR Green qPCR Super Mix-UDG (Invitrogen) and 300 nM of forward and reverse primers. The reactions were performed in the DNA engine Opticon II (MJ Research) with the following settings: 50 C for 2 min, 95 C for 2 min, followed by 45 cycles of 95 C for 15 sec, 55 C for 30 sec, and 72 C for 30 sec. At the end of amplification, a melting curve analysis was done by heating the PCR products to 65–95 C and held for 15 sec at increments of 0.2 C, and the fluorescence was detected to confirm the presence of a single amplification product. Each sample from fed and fasted chickens was run in duplicate to obtain average C_T values for adiponectin mRNA and β-actin mRNA. For negative controls, No-RT controls were used as template in place of single-stranded cDNA in the real-time quantitative PCR. The log-linear threshold values (C_T) during the exponential phase of the PCR for adiponectin mRNA were subtracted from that of β-actin mRNA. Adiponectin mRNA quantity was expressed as a proportion of β-actin mRNA quantity following the $2^{-\Delta\Delta C}{}_T$ method for converting log-linear C_T values to linear term (23). The relative amount of adiponectin mRNA in various tissues were then compared.

Effect of fasting on adiponectin mRNA expression

Chickens were either fed *ad libitum* or fasted (n = 6) for 48 h and killed by decapitation. Liver, adipose tissue from the abdominal fat pad, and anterior pituitary gland were collected from experimental chickens, frozen in liquid nitrogen, and stored at −80 C until analyzed. Brains from the fed and fasted chickens were removed from the cranium, incised coronally at the level of midbrain, frozen on powdered dry ice, and stored at −80 C. Using a cryostat (Richard-Allen Scientific, Kalama-

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                Met Arg Gly Ser Val Gly Phe Leu Leu Cys Ser Leu
   1    ATG AGG GGC TCA GTA GGC TTC CTC CTT TGC TCA CTG
        Leu Leu Ala Leu Ser Gly Thr Glu Met Ala Asp Gln
  37    CTG CTG GCC CTA AGT GGC ACA GAG ATG GCT GAC CAG
        Ala Asp Gln Ser Asp Pro Lys Met Ser Cys Ala Asn
  73    GCT GAC CAG TCC GAC CCC AAA ATG TCA TGT GCC AAC
        Trp Met Gly Gly Ala Pro Gly His Pro Gly His Asn
 109    TGG ATG GGA GGA GCA CCT GGA CAC CCT GGC CAC AAT
        Gly Leu Pro Gly Arg Asp Gly Lys Asp Gly Lys Asp
 145    GGG CTG CCT GGA AGG GAC GGC AAG GAT GGA AAG GAT
        Gly Gln Lys Gly Asp Lys Gly Glu Pro Gly Leu Gln
 181    GGA CAA AAG GGA GAC AAA GGA GAG CCA GGT CTA CAA
        Gly Val Lys Gly Gly Thr Gly Glu Lys Gly Ala Thr
 217    GGT GTC AAA GGG GGC ACG GGT GAA AAG GGT GCC ACA
        Gly Ala Glu Gly Pro Arg Gly Phe Pro Gly His Met
 253    GGT GCA GAA GGA CCG AGA GGA TTT CCA GGA CAC ATG
        Gly Met Lys Gly Gln Lys Gly Glu Ser Ser Tyr Val
 289    GGG ATG AAG GGG CAG AAG GGT GAA AGC TCC TAC GTG
        Tyr Arg Ser Ala Phe Ser Val Gly Leu Thr Glu Arg
 325    TAC CGC TCC GCC TTC AGC GTG GGG CTG ACA GAG CGA
        Ala Pro His Pro Asn Val Pro Ile Arg Phe Thr Lys
 361    GCC CCC CAC CCC AAC GTC CCC ATC CGC TTC ACC AAG
        Ile Phe Tyr Asn Glu Gln Asn His Tyr Asp Ser Ser
 397    ATC TTC TAC AAC GAG CAG AAC CAC TAC GAC AGC AGC
        Thr Gly Lys Phe Leu Cys Ser Ile Pro Gly Thr Tyr
 433    ACC GGC AAG TTC CTC TGC AGC ATC CCC GGC ACG TAC
        Phe Phe Ala Tyr His Leu Thr Val Tyr Met Thr Asp
 469    TTC TTT GCC TAC CAC CTG ACG GTC TAC ATG ACG GAC
        Val Lys Val Ser Leu Tyr Lys Lys Asp Lys Ala Val
 505    GTC AAG GTC AGC CTC TAC AAG AAG GAC AAG GCA GTG
        Ile Phe Thr Tyr Asp Gln Phe Gln Glu Asn Asn Val
 541    ATC TTC ACC TAC GAC CAG TTC CAG GAG AAC AAC GTT
        Asp Gln Ala Ser Gly Ser Val Leu Leu His Leu Ser
 577    GAC CAA GCA AGC GGC TCT GTC TTG CTG CAC CTC AGC
        Leu Gly Asp Glu Val Trp Leu Gln Val Tyr Gly Glu
 613    TTG GGG GAC GAG GTC TGG CTC CAG GTG TAC GGG GAG
        Gly Asn Asn Asn Gly Val Tyr Ala Asp Asn Ile Asn
 649    GGC AAC AAC AAC GGG GTC TAT GCT GAC AAC ATC AAT
        Asp Ser Thr Phe Met Gly Phe Leu Leu Tyr Pro Asp
 685    GAT TCC ACT TTC ATG GGC TTC CTC CTG TAC CCA GAC
        Thr Asp Asp Arg ***
 721    ACA GAT GAC CGT TAG
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FIG. 1. Nucleotide sequence of the chicken adiponectin cDNA open reading frame and the deduced amino acid sequence. The chicken adiponectin cDNA contains 735 bp that translates into 244-amino-acid protein. *Box* represents a portion of the N-terminal end containing 22 glycine-X-Y repeats (in which X and Y represent any amino acid) that are commonly found in the collagenous domain of mammalian adiponectin. Glycine residues within the box are *italicized*. ***, The stop codon.

zoo, MI), brain tissue sections (16 μm) in the coronal plane were made covering the entire diencephalon. Total RNA from the diencephalon tissue sections, liver, and adipose tissue was extracted using Trizol (Invitrogen). Total RNA from the anterior pituitary gland was extracted using RNeasy kit (Qiagen). DNAse-I treated total RNA (1 μg) from each tissue was reverse transcribed as described above. A real-time quantitative RT-PCR analysis was done to quantitate adiponectin mRNA and β-actin mRNA in all of the above tissues using SYBR Green dye as described above. The amount of adiponectin mRNA was expressed as a proportion to β-actin mRNA and compared between fed and fasted chickens.

Statistical analyses and software used

Relative adiponectin mRNA quantity to β-actin mRNA quantity was first converted from log-linear to linear term and then compared using General Linear Models Procedure of SAS (SAS Institute, Cary, NC). Adiponectin mRNA quantity in the adipose tissue, liver, anterior pituitary gland, and diencephalon between fed and fasted chickens was compared using multiple Student's *t* test. A probability level of $P < 0.05$ was considered statistically significant. For DNA sequence analysis and for PCR primer designing, Vector NTI suite 9.1 (Invitrogen) was used.

Results

Cloning of chicken adiponectin cDNA

Using a pituitary cDNA library, we cloned the full-length chicken adiponectin cDNA. The nucleotide sequence of chicken adiponectin cDNA open reading frame is provided in Figure 1. The open reading frame of chicken adiponectin was found to be 735 bp that will yield a protein of 244 amino acids. The chicken adiponectin cDNA is 65–68% homologous to pig, human, mouse, or dog adiponectin cDNA, whereas the deduced protein sequences was 51–61% similar to mammalian adiponectin (Table 1). The deduced amino acid sequence of chicken adiponectin cDNA revealed a series of 22 Gly-X-Y repeat at the N-terminal end, in which X and Y denote any amino acids. Distal to the Gly-X-Y repeats, the amino acid sequences were found to show a slightly higher degree of homology (72.1%) to the globular domain of human adiponectin protein (24). Multiple cysteine residues

TABLE 1. Similarity of chicken adiponectin to mammalian adiponectin

Species	GenBank accession no.	Nucleotide % identity	Amino acid % identity
Dog	AB110099	68	58
Cow	NM174742	67	61
Human	NM004797	66	57
Pig	AY135647	66	51
Mouse	MMU37222	65	60

found in the deduced chicken adiponectin protein may aid in the formation of multimeric forms of adiponectin, as reported with mammalian adiponectin (24).

RT-PCR

A 350-bp product corresponding to nucleotides 250–599 in Figure 1 was amplified from the single-stranded cDNA reverse transcribed from total RNA extracted from adipose tissue, blood, pituitary gland, diencephalon, skeletal muscle, liver, kidney, ovary, and spleen (Fig. 2). A basic local alignment search tool (BLAST) search of the 350-bp chicken adiponectin product revealed significant homology only to mammalian adiponectin and to chicken adiponectin (GenBank accession nos. AY523637 and CD215245) and showed no homology to any other chicken genes. Adiponectin transcript could not be amplified from the blood total RNA (Fig. 2), suggesting that blood cells do not express adiponectin gene. No-RT control samples also did not amplify any product, suggesting that genomic DNA did not contribute to amplification of the adiponectin product (data not shown).

Tissue expression of chicken adiponectin cDNA

Northern blot analysis revealed a single adiponectin mRNA signal (~3.6 kb) in adipose tissue, anterior pituitary gland, liver, diencephalon, skeletal muscle, and kidney but not in the total RNA extracted from blood (Fig. 3).

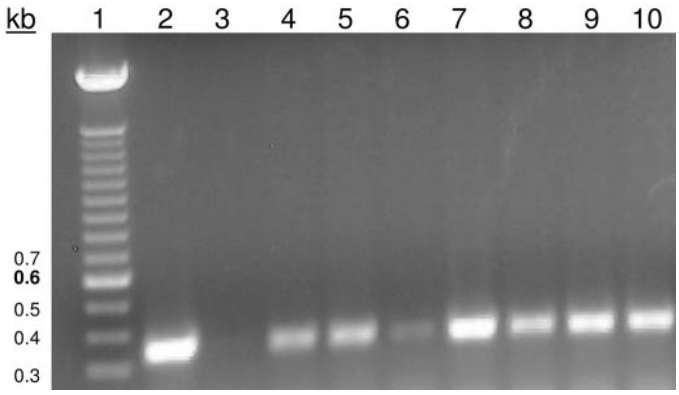

FIG. 2. RT-PCR analysis of adiponectin gene expression in various tissues in the chicken. Total RNA extracted from each tissue was DNAse-I digested and reverse transcribed. Approximately 100 ng of cDNA was used as template to amplify a 350-bp product of chicken adiponectin (nucleotides 250–599 in Fig. 1). Each lane represents one-half of the PCR products from the following tissues: 1, molecular weight marker; 2, adipose tissue; 3, blood; 4, anterior pituitary gland; 5, diencephalon; 6, skeletal muscle; 7, liver; 8, kidney; 9, ovary; 10, spleen.

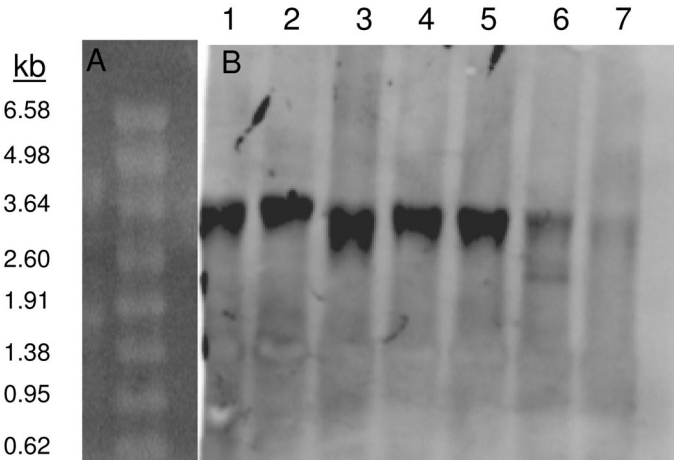

FIG. 3. Northern blot analysis of adiponectin mRNA expression in various tissues in the chicken. Approximately 500 ng of total RNA from each tissue was probed on the Northern blot using a digoxigenin-labeled adiponectin riboprobe (corresponding to nucleotides 250–599 in Fig. 1). After stringency washes, the hybridization signal was detected by alkaline phosphatase-conjugated anti-digoxigenin antibody and ECF chemifluorescent reagent. A, Methylene blue-stained RNA molecular weight standard. B, Northern blot of the following tissues: 1, adipose tissue; 2, anterior pituitary; 3, liver; 4, diencephalon; 5, kidney; 6, skeletal muscle; 7, blood.

Relative quantity of adiponectin mRNA in different tissues

Adipose tissue was found to contain the highest amount of adiponectin mRNA, followed by liver, pituitary gland, diencephalon, kidney, and skeletal muscle (Fig. 4). Melting curve analyses showed the presence of a single PCR product for adiponectin and β-actin products, confirming the specificity of the reaction (data not shown).

Effect of fasting on adiponectin mRNA quantity

Fasting for 48 h resulted in a significant decrease ($P < 0.05$) in the adioponectin mRNA quantity in the adipose tissue,

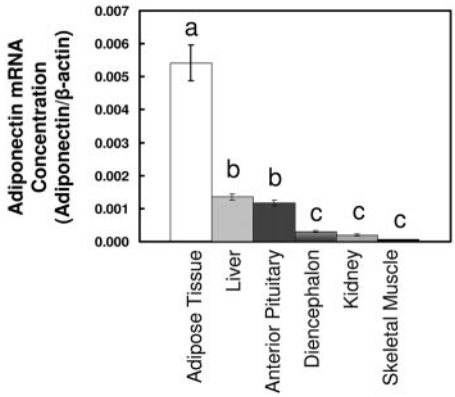

FIG. 4. Adiponectin mRNA quantity relative to β-actin mRNA quantity in various tissues in sexually mature female chicken. Total RNA from each tissue was DNAse-I treated and reverse transcribed. Approximately 50 ng of cDNA was used in real-time quantitative PCR using SYBR Green as the dye to quantitate adiponectin mRNA or β-actin mRNA in separate reactions. Each reaction was run in duplicate, and threshold (C_T) values for adiponectin mRNA were subtracted from that of β-actin mRNA and converted from log-linear to linear term. The data represent mean ± SEM values from three chickens for each tissue. Data with *different letters above each bar* represent significant difference at $P < 0.05$

liver, and anterior pituitary gland compared with *ad libitum* fed chickens (Fig. 5). A decrease in the diencephlaon adiponectin mRNA quantity was not statistically significant ($P = 0.08$).

Discussion

This is the first report to demonstrate adiponectin gene expression in several tissues including adipose tissue and skeletal muscle in any species. We found that the chicken adiponectin gene is expressed in adipose tissue, skeletal muscle, diencephalon, anterior pituitary gland, liver, ovary, and kidney but not in the blood cells. In support of our findings, a chicken skeletal muscle Expressed Sequence Tagged (EST) library contains a partial sequence that is similar to monkey adiponectin cDNA (GenBank accession no. CD215245). In addition, adiponectin expression has been detected recently in the human and swine skeletal muscle (25, 26). Adiponectin gene expression was inducible in human skeletal muscle cells *in vitro* by cell culture supernatants of HEK293 cells transfected with human adiponectin cDNA (27). A brown adipocyte cell line, T37i, was found to express adiponectin mRNA (28). Furthermore, adiponectin mRNA was found recently to be expressed in cultured murine osteoblasts and adiponectin protein secreted into the culture medium (29). The significance of adiponectin gene expression in multiple tissues in the chicken is not known at this time. We find that expression of adiponectin gene in multiple tissues in the chicken is unique and warrants additional investigation. Adiponectin produced in these tissues may supplement circulating adiponectin levels in the blood. Furthermore, adiponectin in these tissues may possibly influence carbohydrate and lipid metabolism in a paracrine or autocrine mechanism. Leptin, another adipocyte-derived hormone, is produced by multiple tissues, such as human placenta, stomach, skeletal muscle, ovary, hypothalamus, and pituitary gland (30, 31).

We determined the relative expression of adiponectin mRNA in various tissues using real-time quantitative RT-PCR and found that adipose tissue was the principal organ in which adiponectin gene is maximally expressed in the chicken as in mammals. Therefore, it is logical to expect adiponectin secretion occurring primarily from adipose tissue in the chicken. The quantities of adiponectin mRNA in other tissues was highest in the liver, followed by the anterior pituitary gland, diencephalon, kidney, and skeletal muscle. Liver is the primary site of lipid biosynthesis (22) in avian species, unlike mammals, in which fat synthesis occurs in white adipose tissue. Adiponectin expressed in the chicken liver may play a regulatory role in lipid metabolism, glucose utilization, and gluconeogenesis. In this regard, intraperitoneal adiponectin administration in mice has been found to decrease both gluconeogenic enzyme gene expression and endogenous glucose production (32).

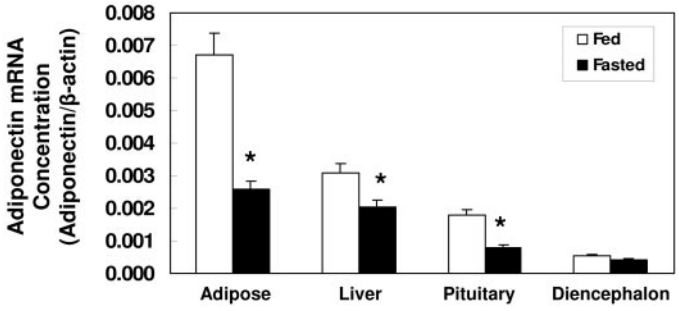

FIG. 5. Effect of fasting on adiponectin mRNA expression in the chicken adipose tissue, liver, anterior pituitary gland, and diencephalon. Chickens were fed *ad libitum* or fasted for 48 h (n = 6) and killed by decapitation. Total RNA was extracted from each tissue and DNAse-I treated. After RT, approximately 200 ng of cDNA was used in real-time quantitative PCR using SYBR Green as the dye to quantitate adiponectin mRNA or β-actin mRNA in separate reactions from fed and fasted chickens. Each reaction was run in duplicate, and the threshold (C_T) values for adiponectin mRNA were subtracted from that of β-actin mRNA, averaged, and converted from log-linear to linear term. Data (mean ± SE) with *different letters above each bar* represent significant difference at $P < 0.05$ between fed and the fasted state.

We found that the chicken pituitary gland and diencephalon expressed greater amounts of adiponectin mRNA next to adipose tissue and liver. Other adipocyte-derived proteins, such as leptin, resistin, and adiponutrin, are expressed in both hypothalamus and anterior pituitary gland in mice (33, 34). Phenotypic identities of diencephalonic neurons or anterior pituitary gland cells that express adiponectin gene in the chicken are not known at this time. In the anterior pituitary gland, adiponectin gene may possibly be coexpressed in GH-secreting cells, based on the finding that plasma GH concentrations are inversely related to adiponectin concentrations in mice (35) and in humans (36). In addition, folliculo-stellate cells in the anterior pituitary gland may possibly express adiponectin mRNA because these cell types have been found to express several other cytokines (37). In the chicken diencephalon, adiponectin gene is possibly expressed in hypothalamic neurons that control secretion of several hormones from the pituitary gland, including GH. It is also likely that adiponectin gene expression in the chicken diencephalon may affect energy homeostasis based on the report that intracerebroventricular administration of adiponectin in mice decreased body weight by stimulating energy expenditure (17).

In the present study, food deprivation resulted in a significant decline in adiponectin mRNA quantity in adipose tissue, liver, and anterior pituitary gland, indicating that adiponectin gene expression is closely linked to energy balance in these tissues. In support of our findings, acute food deprivation decreased adiponectin gene expression in the white adipose tissue of rats (38). Similarly, subcutaneous and epididymal adipose tissue adiponectin mRNA quantity was decreased in response to fasting (39). Fasting decreases gene expression of other adipocyte proteins such as leptin (40), resistin (41), and adiponutrin (42) in the adipose tissue. Anterior pituitary gland expression of other adipose-specific cytokines was found to be affected by food restriction (34). It is likely that fasting would stimulate conservation of energy and therefore attempt to down-regulate expression of adiponectin, a hormone known to promote energy expenditure. It is also possible that a decrease in adiponectin gene expression need not necessarily mean a decrease in adiponectin protein concentration in either the adipose tissue or blood circulation. Such a discordant gene expression and protein expression data have been reported previously for leptin (43). We attempted to detect and quantitate adiponec-

tin protein in the chicken adipose tissue and blood plasma by using an antihuman adiponectin antibody (44) without success. This is possibly due to poor homology (57%) of chicken adiponectin to human adiponectin. To address this question, we are currently making an antibody to a recombinant chicken adiponectin to detect and quantitate adiponectin in blood or in other tissues.

Adiponectin has been reported to act through two receptors (adipoR1 and adipoR2) in the mammalian species (11). We have cloned the chicken homolog of adipoR1 and adipoR2 cDNAs and found them to be expressed in blood cells, diencephalon, anterior pituitary gland, liver, skeletal muscle, spleen, and ovary (Ramachandran R., and S. Metzger, unpublished observation).

In summary, we have characterized chicken adiponectin cDNA sequence and described its unique expression in several tissues in the chicken. We also found that acute fasting significantly decreased adiponectin gene expression in adipose tissue, liver, and anterior pituitary gland. Additional studies are necessary to characterize the role of adiponectin on carbohydrate and lipid metabolism in the chicken.

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Chicken adiponectin cDNA sequence data have been submitted to the GenBank database under accession no. AY838798.

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