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Broiler chicken adipose tissue dynamics during the first two weeks post-hatch

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ABSTRACT

Selection of broiler chickens for growth has led to increased adipose tissue accretion. To investigate the post-hatch development of adipose tissue, the abdominal, clavicular, and subcutaneous adipose tissue depots were collected from broiler chicks at 4 and 14 days post-hatch. As a percent of body weight, abdominal fat increased ($P < 0.001$) with age. At day 4, clavicular and subcutaneous fat depots were heavier ($P < 0.003$) than abdominal fat whereas at day 14, abdominal and clavicular weighed more ($P < 0.003$) than subcutaneous fat. Adipocyte area and diameter were greater in clavicular and subcutaneous than abdominal fat at 4 and 14 days post-hatch ($P < 0.001$). Glycerol-3-phosphate dehydrogenase (G3PDH) activity increased ($P < 0.001$) in all depots from day 4 to 14, and at both ages was greatest in subcutaneous, intermediate in clavicular, and lowest in abdominal fat ($P < 0.05$). In clavicular fat, peroxisome proliferator-activated receptor-γ (PPARγ), CCAAT/enhancer binding protein (CEBP)α, CEBPβ, fatty acid synthase (FASN), fatty acid binding protein 4 (FABP4), lipoprotein lipase (LPL), neuropeptide Y (NPY), and NPY receptor 5 (NPYR5) mRNA increased and NPYR2 mRNA decreased from day 4 to 14 ($P < 0.001$). Thus, there are site-specific differences in broiler chick adipose development, with larger adipocytes and greater G3PDH activity in subcutaneous fat at day 4, more rapid growth of abdominal fat, and clavicular fat intermediate for most traits. Adipose tissue expansion was accompanied by changes in gene expression of adipose-associated factors.

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1. Introduction

Selecting broiler chickens for rapid growth rate has resulted in an increase in voluntary food intake and associated adipose tissue deposition post-hatch (Emmerson, 1997). Adipose tissue is distributed over different body depots, of which abdominal grows the fastest post-hatch in broilers (Fouad and El-Senousey, 2014). The development of adipose tissue occurs as a consequence of both hyperplasia and adipocyte hypertrophy, and thus adipose tissue mass expansion can result from multiplication of new fat cells through adipogenesis and/or from increased deposition of cytoplasmic triglycerides in lipid droplets, as reviewed (Symonds, 2011). To date there have been relatively few reports on the biological process of adipocyte differentiation in chickens, including changes in adipocyte cellularity (Pfaff and Austic, 1976; Hood, 1982; Cherry et al., 1984) and tissue distribution of factors that permit or are necessary for the transition of preadipocytes into adipocytes in vitro (Regassa and Kim, 2013).

In avians, lipogenic activity is greater in the liver than in adipose tissue, and the liver synthesizes very low density lipoproteins (VLDL) from endogenously produced and diet-derived (i.e., chylomicrons) fatty acids and delivers them into the bloodstream (Buyse and Decuypere, 2015). Thus, fatty acids that accumulate in adipose tissue are mostly derived from triacylglycerols in lipoproteins and are then incorporated as triacylglycerols in lipid droplets in the adipocyte. Lipoprotein lipase (LPL) catalyzes hydrolysis of the triacylglyerols from lipoproteins, and as such is a rate-limiting step in adipose tissue lipid accumulation in chickens (Sato et al., 1999). Some of the transcription factors and transcriptional events mediating adipogenesis were identified from studies on rodents (Symonds, 2011) or the 3T3-L1 mouse preadipocyte cell line (Ntambi and Young-Cheul, 2000).

Neuropeptide Y (NPY), a neuropeptide that is abundant in the central nervous system, has received a lot of attention in recent years for its effects on adipose tissue function. It is potently orexigenic and in 5 day-old chicks, central injection of NPY stimulated food intake, with differences in the threshold response between chicks from lines selected for low or high body weight (Newmyer et al., 2013). Neuropeptide Y was also shown to be involved in adipogenesis, using various in vivo and in vitro mammalian models, reviewed by (Zhang et al., 2014), and we reported that NPY promotes adipogenesis in 14 day-old chicken

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adipose-derived cells in vitro (Zhang et al., 2015). In addition to the central nervous system, neuropeptide Y and its receptor sub-types are produced by peripheral tissues, such as the white adipose tissue, skeleton muscle, and liver (Zhang et al., 2013), and NPY and its receptor subtypes NPYR1 and NPYR5 were more highly expressed in the abdominal fat of juvenile high body weight-selected than low body weight-selected line chickens (Zhang et al., 2013).Thus, we hypothesized that the NPY system plays a role in adipogenesis and lipid accumulation in broilers during the early post-hatch period, although the regulation of expression of NPY and its receptors in different tissues during the early post-hatch period has not been reported.

The objective of this study was thus to understand the morphological, biochemical and molecular changes in broiler adipose tissue development during the first two weeks post-hatch.

2. Materials and methods

2.1. Animals

All animal protocols were approved by the Institutional Animal Care and Use Committee at Virginia Tech and animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals. Day of hatch Hubbard × Cobb-500 broiler chicks were obtained from a local hatchery and housed in electrically heated and thermostatically controlled cages with ad libitum access to feed and water. The ambient temperature was gradually decreased from 32 °C on day 1 to 25 °C by 0.5 °C per day, and then 25 °C until 14 days post-hatch. Chicks were fed a standard corn-soybean meal-based commercial starter diet formulated to meet the requirements of Cobb-500 broilers during the starter phase of growth (Cobb-Vantress.com). The formulation was described (Nelson et al., 2015). Both males and females were used for all experiments. At 4 and 14 days post-hatch, randomly selected chicks (n = 6; 3 males and 3 females) were weighed and euthanized for tissue collection, described below.

2.2. Experiment 1: adipose tissue depot weights

Adipose tissue depots, including abdominal (attached to the gizzard), clavicular (discrete mass above the clavicle) and subcutaneous adipose tissue (after peeling back the skin above the cloaca and removing all exposed adipose under the skin), were weighed and weights were converted into a percentage of the chick's body weight. Sex was determined from visual inspection of the gonads.

2.3. Experiment 2: adipose tissue histology

Abdominal, clavicular, and subcutaneous adipose tissues were removed as described above, rinsed in phosphate-buffered saline, submerged in neutral-buffered formalin and incubated overnight at 4 °C. Samples were then dehydrated in a graded ethanol series, paraffin embedded, sectioned at 5 μm, mounted and stained with hematoxylin and eosin. For each tissue, fat was sectioned at three locations, with three sections mounted per slide/location. Images were captured with a Nikon Eclipse 80i microscope and DS-Ri1 color camera, and images analyzed using NIS-Elements Advanced Research Software (Nikon, Melville, NY, USA). Three images were captured on each section, and the density and area of all adipocytes within the field of an image were measured under 20× magnification. The threshold method (Nikon) was used to count adipocytes. Adipocytes were treated as binary objects with the restriction that measurements must exceed 20 μm^2. The mean area and equivalent diameter of each adipocyte and the total adipocyte numbers as well as the total area were recorded. Adipocyte density and the size distribution pattern for each image were also determined.

2.4. Experiment 3: glycerol-3-phosphate dehydrogenase specific activity assays

Abdominal, clavicular, and subcutaneous adipose tissue, pectoralis major, hypothalamus, gastrocnemius, and liver were collected and 0.1–0.2 g of each tissue was transferred to a 10 mL tube containing 5-mL ice-cold lysis buffer (50 mM Tris–Cl, 1 mM EDTA, and 1 mM β-mercaptoethanol, pH 7.5) on ice. The hypothalamus was dissected visually on the basis of the anatomical landmarks as described (Newmyer et al., 2013). The method for assaying G3PDH specific activity was adapted from several studies (Wise and Green, 1979; Lengi and Corl, 2010; Zhang et al., 2015). Tissues were homogenized on ice with a Polytron homogenizer for 30 s at 300 × rpm. This was repeated in 30 s pulses alternating with 30 s on ice twice. The lysates were then centrifuged at 12,000 ×*g* at 4 °C for 30 min, and the supernatant used for measuring G3PDH activity and for determining total protein concentration. The G3PDH activity was measured for each sample in duplicate in assay buffer (100 mM triethanolamine–HCl, 2.5 mM EDTA, 0.12 mM NADH, 0.2 mM dihydroxyacetone phosphate (DHAP), 0.1 mM β-mercaptoethanol, pH 7.5) in a total reaction volume of 200 μL in UV transparent plates (Corning, MA, USA) using a μQuant plate reader and KC Junior software (Bio-Tek, VT, USA). Absorbance was measured at 340 nm for 20 cycles at 25 °C and the maximum slope calculated from the absorbance data. Protein concentration was quantified with Bradford reagent (Sigma-Aldrich, MO, USA) using an Infinite M200Pro multi-mode plate reader and Magellan software (Tecan, CA, USA). The maximum slope was normalized to the protein concentration to calculate specific activity, expressed as μmol/min mg.

2.5. Experiment 4: total RNA extraction and real-time PCR assays

Clavicular adipose tissue, pectoralis major, hypothalamus, gastrocnemius, and liver were collected as described above and snap-frozen in liquid nitrogen and stored at −80 °C until further analysis. Tissues were homogenized in 1 mL Isol RNA Lysis reagent (5-Prime, Gaithersburg, MD, USA) using 5 mm stainless steel beads (Qiagen, Valencia, CA, USA) and a Tissue Lyser II (Qiagen) for 2 × 2 min at 25 Hz. Total RNA was separated following the manufacturer's instructions (5-Prime) and following the step of addition to 70% ethanol, samples were transferred to spin columns and further purified using the RNeasy Mini Kit (Qiagen) with the optional RNase-free DNase I (Qiagen) treatment. The total RNA samples were evaluated for integrity by agarose-formaldehyde gel electrophoresis and concentration and purity assessed by spectrophotometry at 260/280/230 nm with a Nanophotometer Pearl (IMPLEN, Westlake Village, CA, USA).

First-strand cDNA was synthesized from 200 ng total RNA with a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA). Reactions were performed under the following conditions: 25 °C for 10 min, 37 °C for 120 min and 85 °C for 5 min. Primers for real time PCR were designed with Primer Express 3.0 software (Applied Biosystems), and validated for amplification efficiency before use (Table 1). Real-time PCR was performed in duplicate in 10 μL volume reactions that contained 5 μL Fast SYBR Green Master Mix (Applied Biosystems) and 3 μL of 10-fold diluted cDNA using a 7500 Fast Real-Time PCR System (Applied Biosystems). PCR was performed under the following conditions: 95 °C for 20 s and 40 cycles of 90 °C for 3 s plus 60 °C for 30 s. A dissociation step consisting of 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s and 60 °C for 15 s was performed at the end of each PCR reaction to ensure amplicon specificity.

2.6. Statistical analysis

The real time PCR data were analyzed using the ΔΔCT method, where ΔCT = CT target gene − CT actin, and ΔΔCT = ΔCT target sample − ΔCT calibrator (Schmittgen and Livak, 2008). The average of

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