General and Comparative Endocrinology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Research paper

Adipokines expression profile in liver, adipose tissue and muscle during chicken embryo development

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ARTICLE INFO

Keywords:
Adipokines
Chicken
Metabolism
Growth
Fattening

ABSTRACT

In broiler chickens, the intense genetic selection for rapid growth has resulted in an increase in growth rate and fat deposition. Adipose tissue is now recognized as an important endocrine organ that secretes a variety of factors including adipokines. However, the expression pattern of these adipokines is unclear in chicken embryo development. In the present study, we determined the expression profile of three novel adipokines, NAMPT, RARRES2 and ADIPOQ, and their cognate receptors in metabolic tissues (liver, muscles and adipose tissue) of chicken embryo/chicks from 15 days of incubation (E15) to hatching (D0). From E15 to hatching, embryos gradually gained weight and started to develop subcutaneous adipose tissue at E15. We conducted western blot and RT-qPCR tests and found that ADIPOQ expression increased over time and was positively correlated with adipose tissue weight. In addition, NAMPT expression increased only in muscles. By using a new homemade chicken RARRES2 specific antibody we showed that RARRES2 protein levels increased specifically at hatching in adipose tissue, liver and pectoralis major and this was associated with an increase in the weight of embryo. Taken together, these results support a potential involvement of adipokines in metabolic regulation during chicken embryo development.

1. Introduction

The production of broiler chicken has increased over time and was facilitated by intensive genetic selection for rapid growth (Jeong et al., 2011; Lopez et al., 2011). This selection has resulted in changes in body weight and body composition of chicks, including higher proportion of muscle that is the primary source of protein for human consumption. Therefore, the development of animals begins earlier and they reach a body weight around 40 g at hatching and more than 2 kg at 6 weeks old (Goliomytis et al., 2003). Unlike mammals that directly obtain nutrients via the placenta during embryonic development, the energy needed by chickens is provided in two phases. At the beginning of the chickens embryonic development, maternal nutrients are incorporated into the egg yolk during oocyte maturation and are delivered through an expanding vascular system (Burley et al., 1993). Energy expended at this time is mostly produced by glycolysis of accessible glucose. Then, at 14 days of incubation, the embryo is structurally complete, but it still needs very demanding energetic wherewithal to support the last seven days of development (Speake et al., 1998). Thus, considerable metabolic changes occur during this period until hatching, with a switch toward lipolysis (Speake et al., 1998). Around hatching and during the neonatal period, adipose tissue is the main storage site of triacylglycerol, and a transfer of cholesterol ester from the egg yolk to the liver occurs, and in turn, serves as a source of energy. In adult chickens, like humans, but unlike pigs and rodents, the major site for de novo lipid synthesis is the liver rather than adipose tissue (Leveille et al., 1975). Furthermore, chickens present a high level of glycemia (2 g/l), and a low sensitivity to exogenous insulin (Simon, 1989; Braun and Sweazea, 2008). Chickens were often used as a model for studying various metabolic disorders and embryogenesis (Simon, 1989; De Groef et al., 2008; Peng et al., 2018). However, unlike mammals, only limited information is available about the development of adipose tissue (Wang et al., 2017). Therefore, understanding the mechanisms of adipose tissue development in chickens could benefit the poultry industry and

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https://doi.org/10.1016/j.ygcen.2018.06.016
Received 5 March 2018; Received in revised form 31 May 2018; Accepted 23 June 2018

provide new insight into biomedical research.

Adipose tissue is not only a site of lipid storage, but it was also described as an active secretory tissue able to release hormones named adipokines involved in metabolic actions, but also adipogenesis ((Luo and Liu, 2016, Jilal and Devaraj, 2018). Many adipokines were characterized in mammals and associated with body mass index (BMI), food intake and reproductive functions (Dupont et al., 2015; Luo and Liu, 2016). The best-known adipokine is leptin, but its existence in chickens faced a controversial debate (Taouis et al., 1998, Seroussi et al., 2017). In the best of our knowledge, no study was conducted to investigate the expression of other adipokines such as adiponectin (ADIPOQ), visfatin (NAMPT) and chemerin (RARRES2) in chicken embryogenesis. The nucleotide sequences of ADIPOQ, NAMPT and RARRES2 were cloned and those sequences encode for 28, 52 and 16 kDa proteins, respectively (Yuan et al., 2006, Song et al., 2010, Li et al., 2012). ADIPOQ and RARRES2 bind seven transmembrane receptors (ADIPOR1, ADIPOR2, CMKLR1, GPR1, and CCRL2) whereas no receptor was discovered for NAMPT, albeit it activates insulin receptors (Fukuhara et al., 2005). In chickens, NAMPT and ADIPOQ are expressed in various tissues depending on the gender of the animals (Ons et al., 2010, Zhang et al., 2017). In addition, chicken NAMPT is considered more of a myokine than an adipokine (Krzysik-Walker et al., 2008). Concerning chicken RARRES2 and its receptors, we recently demonstrated that they are expressed in liver, adipose tissue and muscle and that RARRES2 plasma levels are related to the fattening of the animals (Mellouk et al., 2018). In mice, ADIPOQ acts as an embryo trophic factor increasing blastocyst cell number and controlling fetal-growth in mice (Cikos et al., 2010, Qiao et al., 2012b, Rosario et al., 2012), but to our knowledge, less was described regarding the role of these adipokines during embryogenesis in chickens. Thus, it becomes crucial to deepen our understanding of the potential involvement of these adipokines as key regulators of metabolic homeostasis during the major growth period of the chicken embryo.

In this present study, we aimed to elucidate the ontogeny of adipokines produced by prenatal metabolic tissues during chicken embryo development. Thus, we characterized the expression profile of adipokines (ADIPOQ, NAMPT and RARRES2) and their receptors in liver, subcutaneous adipose tissue and muscle from the 15th day of incubation (E15) to the day of hatching (D0).

2. Materials and methods

2.1. Ethical issues

All eggs were incubated at the Experimental Unit PEAT 1295 of INRA (Nouzilly, France). The Experimental Unit is registered by the Ministry of Agriculture with the license number D-37-175-1 for animal experimentation. All experiments were approved by the Ethics Committee in Animal Experimentation of Val de Loire CEEA Vdl (permit number 01607.02). The CEEA vdl is registered by the National Committee 'Comité National de Réflexion Ethique sur l'Expérimentation Animale' under the number 19. All experiments were performed in accordance with the European Communities Council Directive 2010/63/UE. Tissues were collected from chicken embryos conventionally incubated at the PEAT experimental unit (INRA, Centre Val de Loire, Nouzilly, France). All the embryos were sacrificed by decapitation and tissues were immediately sampled, snap-frozen and stored at −80 °C until use.

2.2. Animals

Ninety broiler breeder fertile chicken eggs (Cobb 500, Hendrix Genetics, Saint Laurent de la Plaine, France) were incubated according to the following protocol. Eggs were stored in a room at 15–16 °C and 80–85% humidity for one week. Then, all eggs were placed in alternative rows on each shelf of the incubator. They were maintained at 37.8 °C and 56% relative humidity and turned automatically every hour. At day 7 and day 14 of incubation, all eggs were candled and infertile eggs and eggs with dead embryos were eliminated. Three days before hatching, the rotation was stopped, and the temperature was decreased to 37.6 °C. Eggs were then transferred to a compartment in the hatcher. The hatcher was maintained at 36.1 °C and 70% relative humidity. Sixteen fertile eggs were retrieved at fifteen (E15) and nineteen (E19) days of incubation and the last sixteen were retrieved the day of hatching (D0).

2.3. Tissue sampling

Embryos from each stage (E15, E19 and D0) were taken and dissected to collect subcutaneous adipose tissue and liver and muscle (pectoralis major and leg muscle) tissue. Eggs, embryo and subcutaneous adipose tissue were weighed at each stage with a standard balance. Then, tissue samples were frozen in liquid nitrogen and stored at −80 °C until RNA or protein extraction.

2.4. RT-qPCR

Total RNA of 8 embryos per stage was extracted by homogenization in the TRIzol® reagent using an Ultraturax (Invitrogen ™ by Life Technologies ™, Villebon sur Yvette, France) for subcutaneous adipose tissue and extracted using the RNeasy Midi kit (Quiagen®, Courtaboeuf, France) for liver tissue and pectoralis major and leg muscle tissue, according to the manufacturer's recommendations. Concentration and purity of isolated RNA were determined with a NanoDrop Spectrophotometer (Peqlab Biotechnologie GmbH, Erlangen, Germany). The integrity of RNA was checked on 1.25% agarose-formaldehyde gels. The cDNA was generated by reverse transcription (RT) of total RNA (1μg) in a mixture comprising: 0.5 mM each deoxyribonucleotide triphosphate (dATP, dGTP, dCTP, DTTP) 2 M of RT buffer, 15 μg/μL of oligodT, 0.125 U of ribonuclease inhibitor, and 0.05 U MMLV (Moloney murine leukemia virus reverse transcriptase) for one hour at 37 °C. Real-time PCR was performed using the MyiQ Cycle

Table 1
Oligonucleotide primer sequences.

Gene	Product size (bp)	Forward	Reverse	Number of accession	Efficiency
CYTOB	203	5′-CGG ACG AGG CCT ATA CTA CG-3′	5′-GGG AGA ACA TAG CCC ACA AA-3′	KX781319.1	1.95
ß-ACTIN	188	5′-CAG ATG TGG ATC AGC AAG CAG G-3′	5′-TTT CAT CAC AGG GGT GTG GG-3′	NM_001101.4	2
ADIPOQ	64	5′-AAT GTC GTG TGC CAA CTG GAT-3′	5′-TTC CAG GCA GCC CAT TGT-3′	AY786316.1	1.96
NAMPT	96	5′-GCT TCA GCC CAT TTG GTG A-3′	5′-ATC CCG GAA CTG GAT CTT TTG-3′	NM_001030728.1	1.98
RARRES2	71	5′-CGC GTG GTG AAG GAT GTG-3′	5′-CGA CTG CTC CCT AAA GAG GAA CT-3′	NM_001277476.1	1.9
CMKLR1	64	5′-CGG TCA ACG CCA TTT GGT-3′	5′-GGG TAG GAA GAT GTT GAA GGA A-3′	NM_001282407.2	1.96
GPR1	249	5′-ACC TGC CTG AGG AAG AAG AA-3′	5′-AAA GGC CAG TGG AAG CCC AT-3′	XM_004942654.2	1.85
CCRL2	72	5′-CAC GCA GTG TTT GCT TTA AAA GC-3′	5′-CAA CAG CCC ACG TGA CAA TG-3′	NM_001045835.1	1.92
ADIPOR1	349	5′-GAA TAC ACA CCG AGA CGG GC-3′	5′-GCC CAA GAC GCA GAC AAT GG-3′	NM_001031027.1	2
ADIPOR2	344	5′-GAG ACTG GCA ACA TCT GGA C-3′	5′-TGC GAT GCC CAG GAC ACA AA-3′	KP729057.1	1.85