Monitoring the Expression of Leptin Receptor in Avian Model

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Abstract
In this study we carried the expression of leptin receptor in organs and abdominal fat of broiler chickens. The leptin receptor is a member of the class I cytokine receptor family, also known as the gpl30 receptor family, although unlike many other family members, the leptin receptor does not form oligomers with gpl30. Activation of leptin receptors directly or indirectly activates multiple signaling pathways that involve kinase-induced phosphorylation of proteins, including JAK2/STAT3, erbB2, ERK, IRS1 and rho/rac. Signaling requires the presence of intact intracellular domains of the receptor. The leptin receptor is an external tyrosine kinase receptor; upon ligand binding each receptor can bind and activate the tyrosine kinase JAK2, which then cross-phosphorylates tyrosine residues in the other receptor in the dimer. In this study we examined the expression of leptin receptor in organs and abdominal fat of broiler chickens. The aim of our experiment was to optimize the methodology for monitoring the expression of the leptin receptor in avian model. We studied the activity of leptin receptor in the liver, heart, spleen and abdominal fat in the final fattening type broiler chickens Ross 308. In heart tissue, spleen, liver at a relatively high concentration of total cDNA in the sample length leptin receptor fragment located in the expected quantities.

Keywords: broiler chickens, gene expression, leptin receptor, primer pair

1. Introduction

Radical change brought about the discovery of leptin in 1994. Over the next decade, there have been many other secretory proteins - adipokines with endocrine effects, which affect many physiological functions at the whole organism energy balance (appetite - food intake, metabolism of sugars and fats - storage and energy expenditure, thermogenesis) function immune system, reproduction, homeostasis, blood pressure, and angiogenesis. These include proinflammatory cytokines, complement components, fibrinolytic proteins and protein renin-angiotensin system as well as many other bioactive proteins. It is now known for more than 50 adipokines, adipocytes from what makes a cell with a rich and complex secretory function. It is estimated that 20 to 30% of genes expressed in adipose tissue encode secretory proteins precisely [1]. Many of them have local auto-and paracrinic effects on adiposity, growth and differentiation of adipose tissue, adipocyte metabolism and inflammatory processes in adipose tissue, but also endocrine effects in other organs and tissues. The discovery of leptin means a radical shift in understanding the physiological role of white adipose tissue, which actually happened to this discovery the largest human endocrine organs. Leptin (from the Greek Leptos, small or poor) is produce by mature adipocytes. The discovery of leptin is associated with studies in mice with a specific mutation in the gene for leptin, respectively, for the leptin receptor gene. These mutations cause hyperphagia, hyperglycemia, hyperinsulinemic, insulin resistance and abnormal thermogenesis, which leads to massive obesity. It is now best known as leptin "signal saturation" function to maintain body weight within a narrow range by regulation of food intake (anorexigenic effect) and energy output (energy expenditure

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increases sympathetic stimulation of many metabolically active tissues). Central effect of leptin mediated mainly leptin receptor in the hypothalamus. Important are the catabolic effects of leptin in peripheral tissues. In adipose tissue, leptin reduces the cleavage of circulating lipids and the uptake of free fatty acids and glycerol from the circulation and stimulates lipolysis. In addition leptin increases fatty acid oxidation and insulin sensitivity and direct effect on skeletal muscle and liver Creation of leptin in adipocytes regulates the particular state of energy reserves in white fat. Thiazolidinediones and catecholamines inhibit leptin production. Glucocorticoids and insulin are contrary to the production of leptin stimulatory effect. Leptin is therefore a key factor in maintaining energy homeostasis of the organism, which mediates the transfer of information on the status of energy reserves of adipose tissue to the brain. So when in a healthy, lean humans store energy in adipose tissue increases, increased leptin levels provide a negative regulation of energy balance by reducing food intake and increasing energy consumption [2]. It seems that leptin-mediated sympathetic activation in some respects remains unchanged. Increased levels of leptin in this case could contribute to the development of hypertension in patients with metabolic syndrome. Plasma leptin concentrations are independently associated with intima thickness / media arteria carotis communis (an early marker of atherosclerosis). Leptin is also an independent predictor of cardiovascular events in patients with coronary atherosclerosis. It can therefore assume that there is a direct link between hyperleptinemic and increased risk of cardiovascular disease. The pathogenesis of endothelial dysfunction and atherogenesis may apply mitogenic effect of leptin (activation of mitogenic factors, followed by vascular proliferation), sub acute stimulation of vascular inflammation, acceleration trombogenesis as well as increased oxidative stress. Lipodystrophy, characterized by the absence of adipose tissue, like obesity, is characterized by the presence of insulin resistance, diabetes and hypertriglyceridemia. The absence of adipose tissue implies a reduced concentration of adipokines and thus leptin. Leptin deficiency is therefore one of the most important factors in the pathogenesis of metabolic disorders in lipodystrophy and could have some importance in the treatment of metabolic complications of lipodystrophy or generalized lipodystrophy accompanying infection in patients receiving retroviral therapy [3]. Structural analysis suggests that leptin is similar to the cytokine. Ob gene product analysis revealed features that are consistent with the secreted protein acidic and have a high degree of homology between species. For example, human leptin is 84% identical in mouse and 83% identical to ratleptin [4]. Cow and pig leptin is 85% identical to human leptin [5]. This sequence similarity predicts a common function or mechanism of action of this hormone between different species. Was cloned and sequenced chicken leptin cDNA, which is more than 90% identical to Murrin leptin and more than 80% identical to most other known leptin sequences. In addition, leptin cDNA was also cloned turkeys and sequenced and shows very high similarity with sequences of chicken leptin. Chicken leptin has only 145 amino acids compared with 146 amino acids in mammalian species [6, 7]. Another unique structural feature of the chicken leptin is that in comparison with mammalian leptin contains unpaired cytosine at position 3 'of the original cDNA (without signal peptide). Mutation of cysteine did not residue 3 'to the serine residue improve efficacy of chicken leptin [8]. The similarity between the avian leptin and mammalian species remains a subject of debate [6, 9]. A recent phylogenetic analysis showed that the sequence of murine leptin gene have a higher similarity to chicken sequence as in other mammalian sequences [5]. White adipose tissue is considered a key site of leptin production in mammals, but this hormone is also produced in other tissues. Leptin is synthesized in brown adipose tissue in smaller quantities than in white fat [10]. A new dimension of leptin biology had knowledge that the placenta expresses the ob gene and is also the place of production of this hormone [11]. Transcription and translation of leptin gene also occurs in the gastrointestinal tract [12], skeletal muscle [13], mammary epithelium [14], pituitary gland and brain [15]. Hybridization and immunohistochemical studies in pregnant mice in situ synthesis of leptin demonstrated in several areas of the embryo, including the heart, bones, and cartilage and hair follicle cells. While not confirmed, that these tissues synthesize leptin in adults, based on which it is assumed that leptin acts as a growth factor for the fetus and also could

have a role in blood [16]. Leptin messenger ribonucleic acid (mRNA) in chickens is most present in the liver, the largest site of synthesis of fat in birds. In mammals, leptin synthesis in the liver was discovered. The presence of the liver may suggest different mechanisms of expression and leptin production in chickens compared with mammals [7]. Hepatic expression of ob gene in chickens could be explained by an important role in lipid metabolism of the liver, where more than 90% of total lipogenesis is located in the body [17]. Leptin expression was also detected in chicken embryonic liver and yolk sac [7]. Leptin receptor was identified in 1995 along with the Db gene cloning. In rodents, there is a secondary connecting these simple gene products in at least 6 mRNA [13]. One isoform Ob-Rb contains a long intracellular segment, which contains 302 amino acids. In rodents, the Ob-Rb is primarily in the hypothalamus, particularly in the ventre-media and hypothalamus involved in regulating energy intake and energy balance. Ob-Rb mRNA is detected in very small amounts in many peripheral tissues in rodents and humans (liver, small intestine, ovary, spleen, bone marrow, lung, skeletal muscle, kidney and heart) [18]. Four other isoforms of the leptin receptor containing the intracellular domain with 40 amino acids (Ob-Ra, Ob-Rc, Ob-Rd and Ob-Rf, referred to collectively as Ob-RS). Some of these isoforms are found in the brain and affect the regulation of food intake. For example, Ob-Ra is a rich mesh detachment, which may serve to transport leptin into the brain cavity. The presence of Ob-Ra is required to activate the intracellular signal transducer and activator of transcription, but activation of Janus kinase and mitogen-activated protein kinase is also possible without the presence of Ob-Ra [19]. Isoforms of Ob-RS can be found in peripheral tissues in greater quantities than isoform Ob-Rb. Despite their reduced ability signaling may mediate the direct peripheral effects of leptin and may participate in regulating the function of energy, such as gastric emptying, intestinal function, the distribution of nutritional, metabolic oxidation, fetal growth, the responsiveness of the hypothalamus and pituitary and immune function [20]. Last isoform, Ob-Re, contains only the extracellular binding domain. As a result, Ob-Re lacks the ability to signal, but it is important for the dynamics of leptin. Leptin receptor isoforms are examined in detail in rodents, unlike ruminants, which is lack of knowledge. In sheep, leptin is primarily bound to Dorzho - ventromedialis area and hypothalamus [20]. Leptin and its receptors play an important role in metabolism. Many of them have already examined, but several questions remain unanswered, such as the mechanism of action of these genes, as well as regulate their activity in peripheral tissues and in the neuroendocrine system [21]. The focus of this study was to detect extracellular expression of leptin receptor on the analysis of cDNA for the type of final fattening broilers chicken Ross 308. Another aim of study was to focus on the tissue distribution of leptin receptor expression in selected organs broilers chicken.

2. Materials and methods

Analysis was performed on samples, which were obtained from the experimental group with broilers chicken. This experiment was located on poultry farm with a hall designed for holding pieces 24000 broiler chickens for meat production. To detect the leptin receptor, we used tissue samples of internal organs and abdominal fat from broiler chickens Ross 308. Chickens were kept in the hall on deep litter with the recommended conditions and needs of farming. The experiment has been using his technology, feeding and watering. The experimental group was composed of 100 pieces of final fattening type of broilers chicken the Ross 308, from which we chose 10 pieces of equal body weight of 1800 g on end fattening 40 days.

Figure 1. Final fattening of broilers chicken Ross 308. Foto Mrázová (2012)

These broiler chickens were used as representative samples from which we used the internal organs and abdominal fat for analysis. The RNA of isolated sample we crushed in mortar tissue sections of heart, spleen, liver and abdominal fat using the SV Total RNA Isolation System Trial Size kit (Promega, Madison USA). The RNA was transcribed using reverse transcriptase – IMPROM-II™ Reverse Transcription System kit (Promega, Madison USA) with random hexamers as primers. The reaction mixture contained RNA, InPromII 5x buffer, $MgCl_2$, dNTP mix, RNAsin MIX 40pmol.ul^{-1} and InPromII RT. Reaction was following by heating the RNA and Random Hex at 70°C for 5 minutes. Cycle of reverse transcription started at 25°C for 5 minutes. Cycles comprised at 42°C for 50 minutes. This run at 70°C for 15 minutes and then the temperature was lowered to 20 °C for 1 second. Reaction carried out in thermal cycler (PTC-150™ MiniCycler, Research, Watertown USA). This reaction mixture for PCR contained cDNA, 1.80 mM $MgCl_2$, 0.25 mM dNTPs mix. Primer pair used for the analysis was designed in accordance with CASSY et al. [23]. The primers Lep-F (5'-GTCCACGAGATTCATCCCAG-3') and Lep-R (5'-CCTGAGATGCAGAGATGCTC-3') amplified 271 bp long stretch of leptin receptor gene. To confirm the effectiveness of reverse transcription, PCR was also carried out to amplify a cDNA fragment of chicken glyceraldehydes-3-phosphate dehydrogenase (GAPDH) [22]. The primer pair was used GAPDH-F (5'-GTGTTATCATCTCAGCTCCCTCAG) and GAPDH-R (5'- AAAGGTGGAAGAATGGCTGTCACC) amplified 533 bp long stretches. Other components were 0.80 U HotStart Polymerase GoTaq, redestinated water up to volume of 30 ml. Mixture contained buffer GoTaq green 5x buffer (Promega, Madison USA) and amplification was carried out in thermal cycler (PTC-150™ MiniCycler, Research, Watertown USA). PCR reaction procedure was following: PCR cycle started with pre-denaturation at 95°C for 3 minutes. Subsequently, there were repeated 40 cycles comprising denaturation at 95°C for 30 seconds and extension at 72°C for 1 minute. The final extension fragments were at 72°C for 5 minutes. Identification and analysis of isolated DNA was made by electrophoresis in agarose gel. Agarose and 1 x TBE solution were used for preparation of agarose gel. The gel also contained an interaction reagent red (10 µl.ml^{-1}) and ethidiumbromide (2 µl.ml^{-1}). Electrophoresis was performed in 1xTBE solution at a maximum voltage of 5 Vatt and electrode distance (125 V) for 20 minutes. Then the electrode distance was increased to (150 V) for 10 minutes.

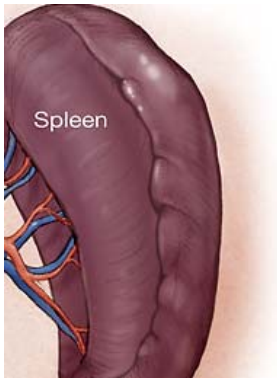

Figure 2. Spleen

Figure 3. Heart

Figure 4. Liver

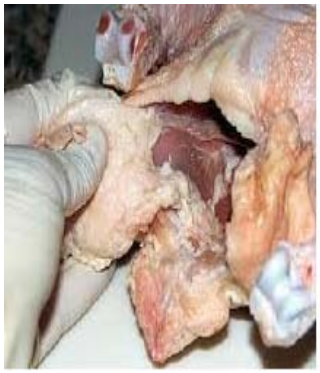

Figure 5. Fat

3. Results and discussion

The obtained total RNA after reverse transcription to cDNA was the template that we used to identify selected leptin receptor. In our experiment, we used weight marker 100 bp in size. As shown in figure 6, in all organs analyzed we were able to isolated total RNA, which in the subsequent reverse transcription and PCR reaction using primer pairs Cassy-F and Cassy-R gave rise to fragments 2, 4, 6 and 8. In all organs analyzed we were able to isolated total RNA, which in the

subsequent reverse transcription and PCR reaction using primer pairs GAPDH-F and GAPDH-R gave rise to fragments 1, 3, 5 and 7. As indicated by the track number 8, the lowest content of isolated RNA was achieved abdominal fat, which we obtained from final fattening type of broilers chicken Ross 308. M is marker for the identification of extracellular leptin receptor.

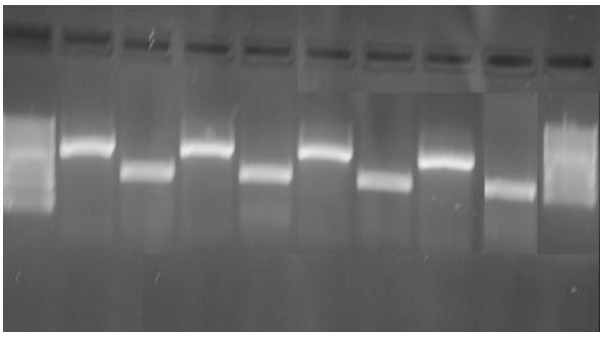

Figure 6. Expression of leptin receptor in internal organs and abdominal fat broiler chickens

M-Weight marker, pathways: 1-spleen tissue control fragment, 2-spleen tissue CASSY fragment, 3-liver tissue control fragment, 4-liver tissue CASSY fragment, 5-heart tissue control fragment, 6-heart tissue CASSY, 7-fat tissue control fragment, 8-fat tissue CASSY fragment.

Interesting results offer track number 8, which should indicate the presence of leptin receptor in abdominal fat. Track of abdominal fat was located fragment, which did not correspond to its length expectancy fragment bounded designed primers (Cassy-F and Cassy-R) fragment length of 273bp [23], but was less than control GAPDH fragment length of 533bp [22]. Unlike Kunová et al [24] that followed leptin in other species of animals for meat production, we have research on leptin receptor in chickens for meat production. The material of searching leptin receptor, we selected internal organs such as heart, liver, spleen and abdominal fat. It follows that, using primers and procedures described in the methodology of work can be studied extracellular expression of leptin receptor in broiler chickens Ross 308. In heart tissue, spleen and liver is studied in leptin receptor expressed in the expected quantities. The tissue abdominal fat appears to be truncated RNA, which could cause alternative splicing molecules (splicing). As the final fattening type Ross 308 has long focused on reducing the fat abdominal fat and increasing the volume of the breast muscle, we will give this issue more.

4. Conclusions

In our experiment, we succeeded isolate total RNA from tissues of internal organs such as heart, spleen, liver, and abdominal fat. In heard tissue, spleen and liver at a relatively high concentration of total cDNA in the sample length leptin receptor fragment located in the expected quantities. Abdominal fat, for the presence of leptin receptor fragment, showed that its length did not correspond to the expected length of the fragment bounded by the proposed ceasefire. Furthermore, we found that using a primer and the described procedures it can be observed extracellular expression of leptin receptor in final fattening type of broilers chicken Ross 308. In body fat tissue is RNA isolated in abbreviated form. The resulting cDNA was determined by UV-spectrophotometric quantification of DNA. This issue we will continue to pay.

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