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Characterization of major elements of insulin signaling cascade in chicken adipose tissue: Apparent insulin refractoriness

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ABSTRACT

The role of insulin in chicken adipose tissue appears weak or questionable. In a first study, proximal and distal components of the insulin signaling cascade were characterized in abdominal adipose tissue of fasted or fed chickens for the first time. Similar measurements were performed on epididymal adipose tissue from fasted or fed rats for comparison. Tyrosine phosphorylation of IR beta subunit, IRS-1 and Shc and phosphorylation of downstream components (Akt and MAPK ERK1/2) were significantly reduced as expected by fasting in rat, but not in chicken. Phosphorylation of MAPK P38 was increased by fasting in chicken but not in rat. Phosphorylation of AMPK was not affected in the conditions investigated in either species. Whatever the nutritional state, the protein levels of IR and IRS-1 were lower in chicken than in rat, whereas those of Shc, Akt, AMPK, MAPK ERK2 and MAPK P38 were similar in both species. In fed state, PI3K activity was higher in chicken than in rat. Insulin sensitivity of insulin cascade was further investigated in chicken adipose tissue following *in vivo* insulin neutralization for 1 or 5 h in fed chickens. Insulin privation did not alter early insulin signaling steps (IRβ, IRS-1 and Shc) or downstream elements (Akt, P70S6K, S6 ribosomal protein, AMPK, MAPK ERK2 and MAPK P38). Finally, phosphorylation of the transcription factor Creb was increased by 2-fold by 5 h fasting or 5 h insulin privation, most likely in response to an increase in plasma glucagon levels. Thus, insulin signaling is markedly different in chicken abdominal adipose tissue from that operating in mammals making chicken an interesting model of insulin resistance or refractoriness.

1. Introduction

In mammals, adipose tissue exerts a crucial feedback in the control of body energy homeostasis by releasing a large number of proteins which signal the status of body energy stores to the brain and peripheral tissues and thereby modulate several physiological functions, such as glucose and intermediate metabolisms and tissue insulin sensitivity. This control is frequently disrupted in humans leading to the development of obesity and diabetes in "modern" societies. Broiler chickens are also subjected to excessive fattening, including during the juvenile period. Chicken lipid metabolism presents several peculiarities. Whereas in rodents, lipogenesis occurs in both adipose tissue and liver [41], in chicken, lipogenesis occurs essentially in the liver [24]; lipogenic enzymes have relatively low activity in avian adipose tissue [18,20]. Thus, in chickens, as in humans, fattening mainly results from the uptake of plasma very low density lipoproteins released by liver. This has been recently emphasized in the model of fattening induced by *in vivo* dexamethasone injections. Exogenous dexamethasone increased the expression of liver acetyl-CoA carboxylase and fatty acid synthase, at least in the fasting state, but had no effect on lipogenic enzyme messengers in adipose tissue. Only messengers encoding lipoprotein lipase were increased in the adipose tissue of fed chickens receiving dexamethasone [5]. On another hand, the regulation of lipolysis in chicken adipose tissue appears largely different from that operating in mammals. In chicken adipocytes lipolysis is almost exclusively regulated by glucagon [23]; the decay of intracellular cAMP levels induced by glucagon is slow

Abbreviations: Akt or PKB, protein kinase B; AMPK, AMP-activated protein kinase; Creb, cAMP response element binding protein; IR, insulin receptor; IRS-1, insulin receptor substrate 1; MAPK ERK1/2, extracellular signal-regulated protein kinase 1/2; MAPK p38, p38 mitogen-activated protein kinase; PI3K, phosphatidylinositol-3′ kinase; Shc, Src homology and collagen protein; S6K1 or P70S6K, 70 kDa ribosomal protein S6 kinase; S6, S6 ribosomal protein.

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[21,27]. On another hand, insulin effects on glucose transport and oxidation in chicken adipocytes, if any, are rather marginal and restricted to cell preparations where lipolytic effects of glucagon appeared normal (see pionner's works reviewed and quoted in [33]. Finally, no anti-lipolytic effect has been demonstrated for insulin in chicken adipocytes (see the corresponding part in [33]). Thus far, only APP (avian pancreatic polypeptide), somatostatin and gut GLI (gut glucagon like immunoreactivity) have been shown to exert an anti-lipolytic effect in isolated adipocytes [22,28,38].

The role of insulin in the control of glucose metabolism in chickens is not yet fully elucidated and still questionable. Chickens have high plasma glucose levels (about 2 g/L) in both the fed and the fasted states and low sensitivity to exogenous insulin [2,33] despite the presence of a hyperactive endogenous insulin circulating at comparatively normal levels [34–36]. Insulin receptor (IR), two IR substrates (IRS-1 and Shc) and several components of the insulin receptor signaling pathways (PI3K, Akt, MAPK ERK2, GSK3, P70S6K and S6 ribosomal protein) have been characterized in chicken liver and muscle under different experimental conditions (fasting and refeeding, chronic corticosterone treatment, genetically fat and lean chicken, exogenous insulin injection and insulin immuno-neutralization) [12,14,9,10,15,16,39,40]. In each situation, the early steps of insulin receptor signaling (IRβ, IRS-1, Shc and PI3K) and downstream elements (Akt, MAPK ERK2, GSK3, P70S6K and S6 ribosomal protein) were accordingly dependent upon insulinemia in liver. In contrast, in muscle, tyrosine phosphorylation of IRβ, IRS-1 and PI3K activity were not affected by any of the experimental conditions. Despite such an apparent insulin refractoriness of early steps of insulin cascade, phosphorylation of Shc and other downstream components were surprisingly insulin sensitive in muscle. Mechanisms permitting a typical regulation of downstream steps in muscle, in the absence of any detectable regulation at the level of IR or proximal steps remain unknown [14].

To our knowledge, the presence of insulin receptors in chicken adipose tissue has been only shown through insulin-crosslinking [1]. In the present study, proximate and distal elements of the insulin signaling cascade have been characterized in chicken adipose tissue and their regulation has been evaluated in response to changes in nutritional status (fed *vs*. fasting status). As external and comparative controls, similar studies were performed using adipose tissue from fasted or fed rats. Finally, the effects of *in vivo* insulin immuno-neutralization were also evaluated in chicken adipose tissue. As recently reported this experimental model induced large changes in several plasma parameters and insulin signaling in liver and muscle [15].

2. Materials and methods

2.1. Animals and experimental conditions

In a first experiment male broiler chickens (Shaver) were housed in individual cages in a conventional controlled-temperature room and daily exposed to 14 h light (06:00–20:00 h) and 10 h dark (20:00–06:00 h) cycles. They were provided with a standard diet and water *ad libitum*. At 5 weeks of age, chickens (1500–1700 g) were divided into two groups of 5 animals. One group was fasted overnight and the other group was maintained *ad libitum* fed. The animals were killed by cervical dislocation. Abdominal adipose tissue was removed, frozen and ground to a powder in liquid nitrogen and stored at −80 °C until use. Male Wistar rats (180–200 g) were used as external and comparative controls. They were either fasted from 09:00 to 14:00 h or *ad libitum* fed ($n = 5$ in each group) and then killed by cervical dislocation. Epididymal adipose tissue was removed, ground to a powder in liquid nitrogen and stored at −80 °C.

Subsequently, the effects of *in vivo* insulin immuno-neutralization were evaluated in chicken abdominal fat tissues. These tissues were issued from the experiment previously described in [15]; the protocol of this experiment is summarized in Fig. 1. Briefly, five groups of seven broiler chickens (ISA 915, Institut de Sélection Animale, Saint Brieuc, France) exhibiting similar body weights (BWs) at 16 or 17 days of age were used. The fed control group received three i.v. injections delivering normal guinea pig serum (PromoCell, Heidelberg, Germany; 1.5 ml/kg each) at time 0, 2 and 4 h (abbreviated as 5hrfed-C group, BW = 475 ± 18 g, $n = 7$). Another fed group received three i.v. injections delivering anti-porcine insulin guinea pig serum (1.5 ml/kg) at 0, 2 and 4 h (abbreviated as 5hrfed-Ab group, BW = 500 ± 22 g, $n = 7$). Immune sera were prepared as described earlier [31,35]. Two additional groups, maintained as fed, received one single i.v. injection (1.5 ml/kg) at 4 h of either normal guinea pig serum (abbreviated as 1hrfed-C group, BW = 505 ± 21 g, $n = 7$) or anti-porcine insulin guinea pig serum (abbreviated as 1hrfed-Ab group, BW = 496 ± 17 g, $n = 7$). The last group, which served as an additional control to measure the extent of changes induced by insulin immuno-neutralization, was fasted for 5 h and given three i.v. injections of normal guinea pig serum (1.5 ml/kg) at time 0, 2 and 4 h (abbreviated as 5hrfasted-C group, BW = 471 ± 18 g, $n = 7$). After blood sampling under EDTA, the chickens were killed by cervical dislocation. Several tissues, including abdominal adipose tissue samples were quickly removed, frozen in liquid nitrogen and stored at −80 °C until analysis. As previously reported, *in vivo* insulin immuno-neutralization induced major changes in several metabolic or endocrine plasma parameters (food intake, glucose, NEFA, aNH2NPN, glucagon and T3) and insulin signaling cascade in liver and muscle [15].

All procedures described herein were approved by the French Agricultural Agency and the Scientific Research Agency and conducted in accordance with the guidelines for Care and Use of Agricultural Animals in Agricultural Research and Teaching.

2.2. Antibodies

Rabbit polyclonal antibodies to MAPK P38 (C-20), MAPK ERK2 (C-14) and phospho-Akt1/2/3 (S473) were from Tebu-Bio (Le

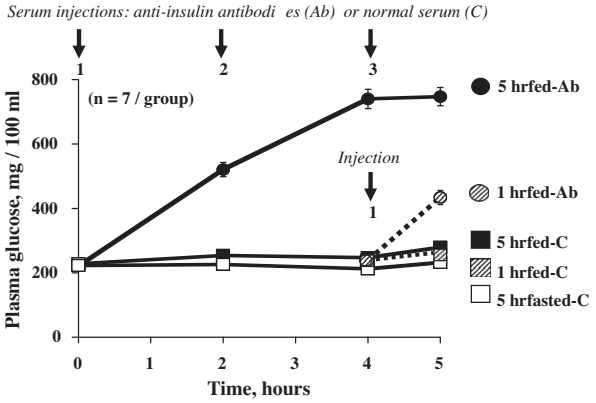

Fig. 1. Experimental design of the insulin immuno-neutralization in 3 week-old male broiler chickens. An experimental group of fed chickens, named "5hrfed-Ab" received 3 intravenous injections of a mixture of anti-insulin antibodies at time 0, 2 and 4 h. A first control group of fed chickens named "5hrfed-C" received 3 intravenous injections of normal guinea-pig serum at the same times. A second control group named "5hrfasted-C" was represented by fasted chickens, starting at time 0; they also received 3 intravenous injections of normal guinea-pig serum at the same time. This fasted group was used to have an estimate of changes induced in another model of insulin privation. Birds were killed at time 5 h that is 1 h after the third serum injection. Two other groups of fed chickens (dotted lines) received only one serum injection at time 4 h: the 1hrfed-Ab group received anti-insulin serum and the 1hrfed-C group received normal serum. These two groups were killed at 5 h, i.e., 1 h after the unique injection in 1hrfed-Ab and 1hrfed-C groups.