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FAT RESISTIN AND ADIPONECTIN mRNA EXPRESSION IS MODULATED BY GLUCOCORTICOIDS IN MICE

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It is known that obesity is associated with a state of chronic low-level inflammation. Some data indicate that obesity-related stress could increase the hypothalamo-pituitary-adrenal axis activity. This leads to a rise in the plasma glucocorticoid level, which induces the development and differentiation of preadipocytes. Thus, the present study was performed to examine the effect of glucocorticoids on the expression of resistin and adiponectin mRNAs in adipose tissue during acute and chronic inflammation in mice. The results of the study showed an increase in the expression of resistin mRNA and a decrease in the expression of adiponectin mRNA during chronic inflammation (obese animals). Synthetic glucocorticoids changed the expression of both adipokines in a different manner according to the state of inflammation. To sum up, the action of glucocorticoids in adipose tissue depends on the immune system activity.

Key words: resistin, adiponectin, inflammation, glucocorticoids, mice

INTRODUCTION

White adipose tissue (WAT) plays an important role in maintaining systemic energy balance by acting as a reservoir for excess energy, and it is an important target for both natural and synthetic glucocorticoids (AHIMA and FLIER, 2000). Glucocorticoid receptors are involved in metabolic regulation and distribution of body fat (JOYNER et al., 2000). They show regional variation in density with elevated concentrations in WAT (REBUFFÉ'-SCRIVE et al., 1985).

In addition, mature adipocytes act as an active endocrine and paracrine organ and through a communication network with other tissues, sympathetic nervous system and brain can influence appetite, energy balance, immunity, insulin sensitivity, angiogenesis, blood pressure, lipid metabolism and homeostasis (CHALDAKOV et al., 2003). Adipocytes contribute to the raised proinflammatory state in obesity and diabetes. They are capable of synthesizing pro-inflammatory and anti-inflammatory proteins (HAUNER, 2005). Resistin and adiponectin, the two important adipokines produced by mature adipocytes, have wide physiological effects mainly on inflammation modulation. Resistin was identified as a 12.5 kDa polypeptide expressed and secreted by white adipose tissue.

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The term "resistin" was originally proposed for its role as a mediator of insulin resistance (Steppan et al., 2001). Some studies showed that resistin can also play a role in inflammation and autoimmunity. Pro-inflammatory cytokines, such as IL-1, IL-6 and TNF, as well as LPS strongly induced resistin mRNA expression (Kaser et al., 2003). On the other hand, in humans, resistin enhanced the secretion of pro-inflammatory cytokines, TNF and IL-12, and was able to induce the nuclear translocation of NF-kappaB transcription factor (Silswal et al., 2005). Adiponectin is another adipokine extensively secreted from mature adipocytes that sensitizes various organs to insulin action (Wang et al., 2008). Furthermore, this adipokine is also implicated in the pathophysiology of some inflammatory and immune response processes; it induces the production of important anti-inflammatory factors, such as IL-10 (Matarese et al., 2005; Fantuzzi, 2008). Adiponectin is a metabolically active adipokine that is inversely associated with obesity, insulin resistance and atherosclerosis. A lot of studies indicated that adiponectin has anti-inflammatory, antiatherogenic and antidiabetic properties (Matsuzawa, 2005).

Glucocorticoids are steroid hormones that play a role in regulating systemic energy metabolism and modulating immune and inflammatory processes. Because of their anti-inflammatory properties, synthetic glucocorticoids are used therapeutically for both acute and chronic inflammatory and immune disorders. Most tissues are targets for glucocorticoids and contribute to a wide range of physiological effects of these hormones.

Data from the literature suggest that glucocorticoids regulate the expression of adipokines at the mRNA and protein levels, but most of these investigations are either *in vitro* studies on isolated adipocytes or single points, or *in vivo* studies on rodent models.

Thus, in this study we planned to examine the effect of glucocorticoids on the mRNA expression of two opposing proteins, resistin and adiponectin in adipose tissue during acute and chronic inflammation in mice.

MATERIALS AND METHODS

Animals

The experiment was carried out on 6-week-old Swiss mice (female, n=24), maintained at a constant temperature of 23°C±1 with a 12 h light/dark cycle and given water *ad libitum*. The animals were divided into two groups: non-obese mice (fed a commercial food, BW=27±1.4g) and obese mice with chronic inflammation (fed a high-calorie diet for 3 weeks, BW=33±1.9g). In each group mice were divided into 4 experimental subgroups: I – control, II- acute inflammation (STZ), III- treated with glucocorticoids (DEX), and IV- STZ with glucocorticoids. The animals from the control group received an intraperitoneal (i.p.) injection of saline. To develop acute inflammation, the animals received injections (i.p.) of STZ (Streptozotocin, Sigma-Aldrich). The mice were weighed prior to injection, and STZ was freshly dissolved in dilution buffer (0.1 M sodium citrate, pH 4.5, with HCl, stored at 4°C). STZ was given at a dose of 0.2 ml (single i.p. injections of 100 mg STZ/kg of body weight), 24 hours before decapitation. Mice from subgroup III were treated with dexamethasone (synthetic glucocorticoid, 0.2 ml, i.p.) two times - 24 h and 1 h before decapitation. After injections the animals were decapitated and white adipose tissue (WAT) was quickly removed and stored in RNA Later (-20°C) for further analysis.

RNA extraction and cDNA synthesis

Total RNA was extracted from WAT using Trizol Reagent (Life Technologies, USA) with our own modification. Concentration and purity of the RNA samples were determined by UV spectroscopy at 260/280 nm, and integrity was confirmed by electrophoresis through 1% agarose gels stained with ethidium bromide. The first strand cDNA was transcribed from 1 µg RNA with MultiScribe Reverse Transcriptase (50U/µl, Life Technologies, USA) using random primers at 25°C for 10 min, and then at 37°C for 120 minutes and 85°C for 5s. The cDNA was reconstituted in 50 µl of sterilized water and 100 ng of the cDNA solution was used as a template.

Quantitative PCR

Quantitative PCR analysis was performed using the StepOnePlus Real-Time PCR System (Life Technologies, USA) with the Universal Master Mix and TaqMan chemistry (Life Technologies, USA).

The reactions were as follows: initially 50°C for 2 min, then 95°C for 10 min, to activate the AmpliTaq Gold DNA polymerase, and next 40 cycles of 95°C for 15 s and 60°C for 1 min. The data obtained for resistin and adiponectin were compared with the data obtained from amplification of the reference gene 18sRNA (Life Technologies,USA). The adipokine expression was assessed using a designed gene expression assay (Life Technologies, USA).

The $2^{-\Delta\Delta C}_{t}$ method with 18sRNA as an internal control and one of the control mice as an internal calibrator was used to present a change in gene expression.

The results were expressed as mean ± SD. Comparisons of the group means were made using Student's *t*- test. P<0.05 was considered statistically significant.

RESULTS

Resistin mRNA gene expression (Fig.1)

The prolonged treatment with a high-calorie diet significantly increased (by 300%) the expression of resistin mRNA in mice adipose tissue (obese animals) as compared with mice (lean animals) fed a standard diet (4.07±1.1 resistin/18sRNA vs. 1.0±0.08 resistin/18sRNA, P<0.01). A single streptozotocin injection induced inflammation and decreased the expression of resistin in both the experimental groups (lean and obese mice) in a similar manner (by 40%). In contrast, the treatment with immunosuppressive synthetic glucocorticoid stimulated resistin mRNA expression in mice fed a standard diet (4.19±1.06 resistin/18sRNA, by 320%, P<0.01), and inhibited it in obese animals (by 87%, P<0.05). Dexamethasone and streptozotocin given together decreased resistin expression in all the animals (independently of the diet used).

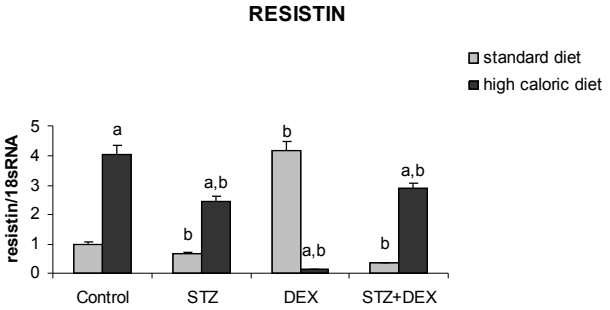

Fig.1. Expression of resistin mRNA in visceral adipose tissue (X±SE, [a]P<0.05-0.01 – compared with a standard diet, [b]P<0.05-0.01 – compared with the control group)

Adiponectin mRNA gene expression (Fig.2)

The obtained results indicate that adiponectin mRNA expression was decreased in obese animals as compared with lean mice (0.45±0.06 adiponectin/18sRNA vs. 1.0±0.09 adiponectin/18sRNA, P<0.01). Decreased expression of adiponectin during acute inflammation was observed only in lean animals (by 62%, P<0.01). Dexamethasone increased the expression of adiponectin in both the experimental groups (by 35% in mice fed a standard diet and by 60% in mice fed a high-calorie diet, P<0.05).

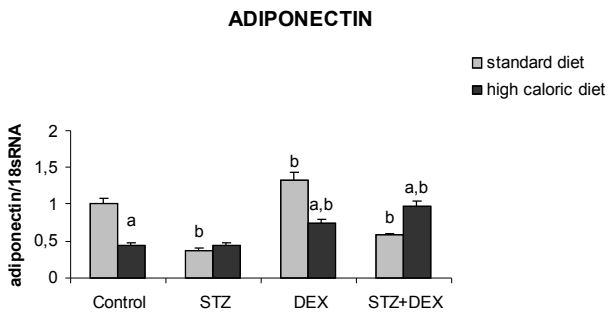

Fig.2. Expression of adiponectin mRNA in visceral adipose tissue (X±SE, [a]P<0.05-0.01 – compared with a standard diet, [b]P<0.05-0.01 – compared with the control group)

DISCUSSION

The present experiment was performed to determine the influence of short-term peripheral glucocorticoid administration on local resisitin and adiponectin mRNA expression in visceral adipose tissue in mice fed standard and high-calorie diets. The results of the current study demonstrate increased expression of resistin and decreased expression of adiponectin in obese mice. These findings are consistent with the results of earlier studies which demonstrated that adipose tissue increased the expression of multiple genes, including resi-

stin gene expression, as a result of high-fat diet-induced obesity in rats (Li et al., 2002). It was also demonstrated that calorie restriction increased gene expression and blood levels of adiponectin in rodents (Combs et al., 2003). White adipose tissue has been strongly linked to the pathophysiology of many metabolic disorders which are also some of adverse effects of long-term glucocorticoid treatment (Wang et al., 2008). Adiponectin and resistin belong to the group of adipocytokines which are thought to be involved in regulation of insulin sensitivity, and which also exert opposite effects on inflammatory processes related to atherosclerosis and cardiovascular risk. Glucocorticoids, besides possessing anti-inflammatory properties, also increase insulin resistance. It was therefore of interest to determine the effect of these steroids on the expression of visceral adipose tissue adipokines. The present study has shown a stimulating effect of synthetic glucocorticoid (dexamethasone) on resistin mRNA expression in mice fed a standard diet, and an inhibiting effect in obese animals. We have also observed upregulation of adiponectin mRNA expression after dexamethasone injection, both in mice fed a standard diet and in those fed a high-calorie diet. Some earlier studies on the effect of glucocorticoid treatment on adiponectin expression showed contradictory results dependent on the origin of the steroids used. Shi with co-workers showed that hydrocortisone treatment of both obese and normal rats decreased adiponectin expression, which was also reflected in its plasma concentrations (Shi et al., 2010). On the other hand, dexamethasone given to both normal and hypoxic rats and humans increased plasma adiponectin (Jang et al., 2008). In our study interesting results were obtained in the groups of animals receiving both streptozotocin and glucocorticoid which differentially affected the tested adipokines. The drug combination decreased resistin expression in all the animals (independently of the diet used), however adiponectin expression was decreased only in lean mice. Obese mice treated with dexamethasone and streptozotocin showed a significant increase in adiponectin mRNA expression. It can be concluded that the therapeutic strategy for glucocorticoid treatment should consider the differentiated activity of fat adipokines.

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