Increased resistin expression in the adipose tissue of male prolactin transgenic mice and in male mice with elevated androgen levels

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Abstract The aim of this study was to investigate the regulation of resistin, a recently identified adipocyte-secreted peptide, in the adipose tissue of prolactin (PRL)-transgenic (tg) mice using ribonuclease protection assay. The level of resistin mRNA increased 3.5-fold in the adipose tissue of untreated male PRL-tg mice compared to controls. However, there was no difference in resistin expression in the adipose tissue of female PRL-tg mice compared to control mice. PRL-tg male mice have elevated serum testosterone levels and we therefore analyzed the effects of testosterone alone on resistin mRNA expression. Furthermore, the effects of elevated androgen levels on PRL receptor (PRLR) mRNA expression in the adipose tissue were investigated. Resistin mRNA increased 2.6-fold in the adipose tissue of control male mice with elevated serum androgen levels. In addition, PRLR mRNA expression was increased in the adipose tissue of male mice with elevated testosterone. These results suggest testosterone to be a regulator of resistin and PRLR mRNA expression in the adipose tissue of male mice.

Key words: Resistin; Prolactin; Testosterone; Adipose tissue; Liver; Prolactin receptor

1. Introduction

A new adipocyte-secreted signaling molecule, resistin, was recently identified [1,2]. Resistin was found to antagonize insulin's action in rodents [1]. Mice treated with recombinant resistin became glucose intolerant and insulin resistant compared to vehicle-treated mice. In addition, neutralization of resistin, using an anti-resistin antibody, enhanced insulin-stimulated glucose uptake by adipocytes in vitro [1]. Furthermore, circulating resistin levels increased in mice with diet-induced and genetic forms of obesity, while resistin levels were decreased by thiazolidinediones (TZDs) [1]. In contrast, a different study demonstrated that resistin mRNA expression was decreased in the epididymal adipose tissue of obese ob/ob, db/db, tub/tub and KKA^{y} mice and Zucker diabetic fatty rats compared to their age-matched lean littermates [3]. Fasshauer et al. have investigated the effects of isoproterenol, dexamethasone and triiodothyronine on resistin mRNA expression in 3T3-L1 adipocytes in vitro and they demonstrated that isoproterenol inhibits resistin expression via a Gs-protein-coupled pathway [4].

Several studies indicate that prolactin (PRL) is a diabetogenic hormone and hyperprolactinemia induces glucose intolerance, hyperinsulinaemia and insulin resistance in both human patients and rodents [5–12]. The PRL receptor (PRLR) is a member of the cytokine receptor superfamily and it mediates the effects of PRL on its target tissues [13]. In a recent study, we demonstrated PRLR expression in mouse adipocytes [14]. In addition, PRLR expression increased in the adipose tissue during lactation and in female and male PRL-transgenic (tg) mice compared to controls [14].

So far, there is limited information concerning how PRL induces insulin resistance. Because resistin has been demonstrated to antagonize insulin's action in rodents, in the present study we analyzed the effects of hyperprolactinemia on resistin mRNA expression in the adipose tissue of male and female PRL-tg mice. Furthermore, male PRL-tg mice have elevated androgen levels [15] and the effect of increased serum testosterone on resistin expression was therefore examined. In addition, we examined the independent and combined effects of elevated serum PRL and testosterone levels on regulation of PRLR mRNA expression in the adipose tissue and liver of male PRL-tg and control mice.

2. Materials and methods

2.1. Animals

Heterozygous male and female PRL-tg mice, over-expressing rat PRL (rPRL), and non-tg littermates, control mice, were used in this study [15,16]. All mice were kept in a controlled environment with free access to water and pelleted food. The animal experiments were approved by the local ethics committee. At 12 weeks of age, male mice were castrated via the abdominal route and slow release placebo or testosterone pellets were implanted subcutaneously in the upper dorsal part of the neck. According to group, male mice received either 7.5 mg (T7.5) or 30 mg (T30) testosterone or placebo pellets (Innovative Research of America, Sarasota, FL, USA). 8 weeks after castration and pellet implantation, male PRL-tg and control mice were euthanized by heart puncture under general anesthesia (ketalar/rompun). Epididymal adipose tissue, liver and serum samples were obtained from all male mice. Parametrial adipose tissue was obtained from all female mice. All tissues were fresh-frozen in liquid nitrogen and stored at −70 until the time of RNA preparation.

2.2. RNA analysis

Total RNA was isolated from frozen tissues using Tri reagent according to the manufacturer's instructions (Sigma, St. Louis, MO, USA). Mouse resistin, long PRLR isoform (L-PRLR) [17] and cyclophilin antisense RNA probes were used in a ribonuclease protection

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Abbreviations: PRL, prolactin; RPA, ribonuclease protection assay; TZDs, thiazolidinediones; RT-PCR, reverse transcription-polymerase chain reaction; tg, transgenic

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assay (RPA). A 365-bp mouse resistin cDNA fragment (nucleotides 145–509 [1]) generated by reverse transcription-polymerase chain reaction (RT-PCR) using resistin-specific primers (upstream primer: 5′-CCAGCATGCCACTGTGT-3′, downstream primer: 5′-GTAGAGACCGGAGGACATCA-3′) was subcloned into the pCRII-TOPO vector (Invitrogen, Carlsbad, CA, USA). The resistin construct was verified by DNA sequencing and used as a template for making an antisense RNA probe. A 288-bp mouse L-PRLR cDNA fragment (nucleotides 1507–1794) subcloned into a pBluescript vector was used as a template [14]. A 103-bp mouse cyclophilin probe (nucleotides 38–140, Ambion, Austin, TX, USA) was used as an internal standard to control the amount of RNA in each sample. The RPA was performed using the RPAIII™ RPA kit (Ambion). RNA samples were hybridized with antisense [^{32}P]CTP-labeled cyclophilin-RNA probe together with either antisense [^{32}P]CTP-labeled resistin-RNA probe or L-PRLR-RNA probe. Protected fragments were separated on a denaturing 8-mmol/l urea/6% polyacrylamide gel (Novex, San Diego, CA, USA). Dried gels were exposed to a phosphorimager screen, developed in a phosphorimager (Molecular Dynamics Inc., Sunnyvale, CA, USA), and quantitative analysis was performed using ImageQuant software (Molecular Dynamics Inc., Sunnyvale, CA, USA). Resistin/cyclophilin and L-PRLR/cyclophilin ratios were calculated for the respective samples and the average ratio for the control mice was set at 100% for resistin and L-PRLR, respectively.

2.3. Measurement of testosterone

After 8 weeks of treatment, serum testosterone levels were measured in male mice by double antibody RIA according to the manufacturer's instructions (ICN Biomedicals, Costa Mesa, CA, USA) (Table 1). Serum testosterone levels were significantly elevated in untreated male PRL-tg mice and in male control mice implanted with a 30 mg testosterone pellet (T30) compared to control mice. Furthermore, castrated male PRL-tg and control mice implanted with 7.5 mg testosterone pellets (T7.5) had normophysiological testosterone levels compared to control mice. In addition, testosterone was not detected in serum from castrated male mice implanted with placebo pellets.

2.4. Statistical analysis

Differences in resistin and L-PRLR mRNA expression (comparing resistin/cyclophilin or L-PRLR/cyclophilin ratios) and serum testosterone concentrations were analyzed using one-way ANOVA followed by the Student–Newman–Keuls multiple range test.

3. Results

The effects of chronically high PRL levels on resistin mRNA expression were investigated in the adipose tissue isolated from female and male PRL-tg mice and controls using RPA (Fig. 1A,B). The level of resistin mRNA expression increased 3.5-fold in the adipose tissue of untreated PRL-tg male mice compared to control mice (Fig. 1B). However, no significant difference was detected in resistin expression in the adipose tissue of female PRL-tg mice compared to controls (Fig. 1A). Male PRL-tg mice, over-expressing rPRL, have previously been reported to have elevated serum testosterone levels compared to control mice [15]. In order to study the effects of elevated PRL levels and androgen levels independently, 12 weeks old male PRL-tg and control mice were castrated and placebo or testosterone (T7.5 or T30) pellets were implanted (Table 1). Animals were treated for 8 weeks. The level of resistin mRNA was increased (2.6-fold) in control male mice with elevated testosterone levels, control +T30 (Fig. 1B). In order to determine whether PRL in the absence of testosterone or in combination with normophysiological

Table 1
Serum testosterone levels in 5 month old male PRL-tg and control mice after 8 weeks treatment

Treatment group	Testosterone (nmol/l)
Control	4.27 ± 1.56
PRL-tg	23.74 ± 2.67*
Control: castrated + placebo	Not detected
PRL-tg: castrated + placebo	Not detected
Control: castrated +T7.5	9.33 ± 1.28
PRL-tg: castrated +T7.5	7.39 ± 1.73
Control +T30	24.12 ± 3.26*

Values are the mean ± S.E.M. (4–8 mice/group) and statistical significance assessed using one-way ANOVA followed by Student–Newman–Keuls multiple range test. *$P < 0.05$ vs. control.

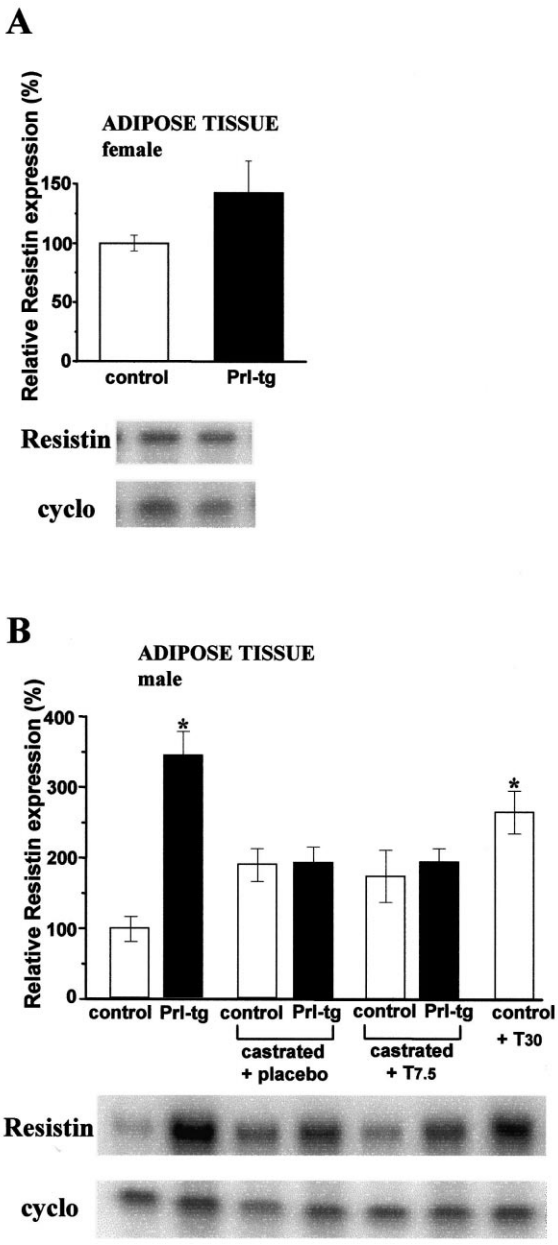

Fig. 1. Expression and regulation of resistin mRNA in parametrial adipose tissue of female (10 mice per group, $P = 0.090$) (A) and in epididymal adipose tissue of male (4–8 mice per group) (B) PRL-tg (tg) and control mice receiving treatment as described in Section 2. The resistin/cyclophilin ratio was calculated for each sample and the values used as the relative resistin expression (the average value for the controls was set to 100%). Results are expressed as mean ± S.E.M. and statistical significance assessed using one-way ANOVA followed by Student–Newman–Keuls multiple range test. *$P < 0.05$ vs. control.

testosterone levels affects resistin expression, resistin mRNA was analyzed in the adipose tissue of castrated male PRL-tg mice implanted with either placebo or T7.5 pellets. The level of resistin mRNA was unchanged in the adipose tissue of castrated PRL-tgs implanted with either placebo or T7.5 pellets (Fig. 1B). In addition, there was no difference in resistin mRNA expression in the adipose tissue of castrated control male mice implanted with either placebo or T7.5 pellets.

Furthermore, to determine whether the level of L-PRLR expression in adipose tissue is regulated by the elevated testosterone levels found in untreated PRL-tg male mice, L-PRLR mRNA expression was analyzed in untreated PRL-tg males and in castrated PRL-tg male mice implanted with placebo or T7.5 pellets. The level of L-PRLR was increased 3-fold in the adipose tissue of both untreated and castrated PRL-tg male mice compared to control mice (Fig. 2). However, the increased L-PRLR expression found in PRL-tg male mice was not affected by the different testosterone levels in untreated or castrated males implanted with placebo or T7.5 pellets. Nevertheless, in the adipose tissue of mice with elevated testosterone levels, control male mice implanted with T30 pellets, L-PRLR mRNA expression increased 2-fold compared to untreated controls (Fig. 2). Furthermore, there was no difference in L-PRLR expression in the adipose tissue of castrated controls with placebo or T7.5 pellets.

The regulation of L-PRLR mRNA in the adipose tissue of PRL-tg male mice was compared with its regulation in the liver (Fig. 3). The level of L-PRLR mRNA was increased in the liver of both untreated and castrated PRL-tg male mice compared to untreated control mice. However, L-PRLR mRNA increased more in castrated placebo-treated PRL-tg males, compared to PRL-tgs with testosterone, the untreated or the T7.5 pellet-treated males (Fig. 3). In addition, L-PRLR

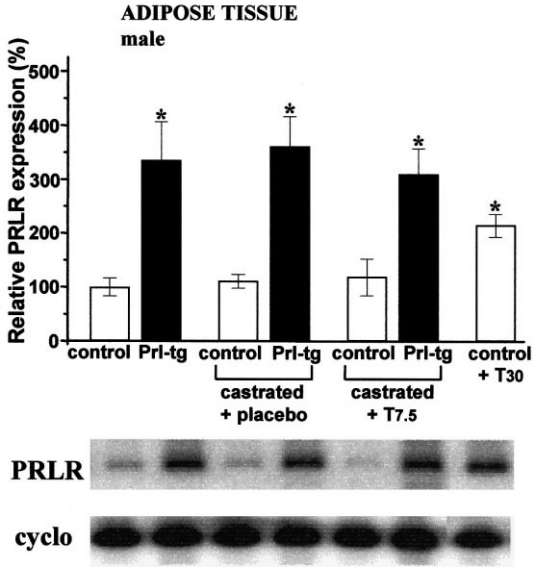

Fig. 2. Expression and regulation of L-PRLR mRNA in epididymal adipose tissue of male PRL-tg and control mice receiving treatment as described in Section 2. The L-PRLR/cyclophilin ratio was calculated for each sample (4–8 mice per group) and the values used as the relative L-PRLR expression (the average value for the controls was set to 100%). Results are expressed as mean ± S.E.M. and statistical significance assessed using one-way ANOVA followed by Student–Newman–Keuls multiple range test. $*P < 0.05$ vs. control.

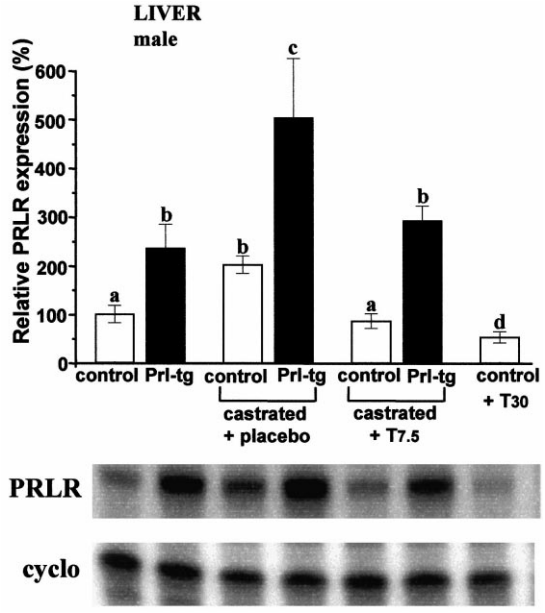

Fig. 3. Expression and regulation of L-PRLR mRNA in the liver of male PRL-tg and control mice receiving treatment as described in Section 2. The L-PRLR/cyclophilin ratio was calculated for each sample (4–8 mice per group) and the values used as the relative L-PRLR expression (the average value for the controls was set to 100%). Results are expressed as mean ± S.E.M. and statistical significance assessed using one-way ANOVA followed by Student–Newman–Keuls multiple range test. Bars with different superscripts are significantly different from each other ($P < 0.05$).

mRNA expression increased in the liver of castrated placebo-treated control male mice compared to castrated T7.5 pellet-treated, untreated and T30 pellet-treated control mice. The lowest hepatic L-PRLR expression was found in control male mice with elevated serum testosterone levels implanted with T30 pellets.

4. Discussion

PRL has been found to induce glucose intolerance and insulin resistance in several species [5–12]. A new adipocyte-secreted peptide, resistin, was recently identified [1,2]. Resistin was proposed to represent a link between obesity and diabetes. No resistin-receptor has so far been identified but resistin was found to impair glucose tolerance and insulin action in rodents. The present study demonstrates increased resistin mRNA expression in the adipose tissue of untreated PRL-tg male mice. The regulated resistin expression might be a link between elevated serum PRL levels and altered glucose metabolism. However, there was no difference in resistin expression in the adipose tissue of female PRL-tg mice compared to controls. In addition to elevated serum PRL levels, male PRL-tg mice also have elevated testosterone levels. The reason for this is that PRL stimulates LH-receptor expression in the testicular Leydig cells of rodents, resulting in increased testosterone production [13,18]. When the level of serum testosterone was normalized or eliminated in PRL-tg males, resistin mRNA expression was unchanged compared to control mice. This observation suggests that for resistin expression to increase in PRL-tg male mice, testosterone needs to be elevated. Therefore, we investigated the effects of prolonged elevated testosterone treatment on resistin expression in control mice.

Resistin mRNA expression increased in the adipose tissue of mice with elevated serum testosterone concentrations. However, the induction of resistin mRNA was stronger in untreated PRL-tg mice compared to mice where only serum testosterone was elevated.

The regulation of the PRLR is under hormonal control and the expression of the PRLR has for an example been shown to vary as a function of the stage of the estrous cycle, pregnancy and lactation [13]. In a recent study, our group demonstrated increased levels of L-PRLR mRNA in both the adipose tissue and the liver of female and male PRL-tg mice [14]. These findings demonstrate an important role for PRL in regulating PRLR expression and possibly the metabolism in the adipose tissue and the liver. However, because male PRL-tg mice also have elevated androgen levels, we investigated the independent and the combined effects of elevated PRL levels and androgen levels on PRLR expression in both the adipose tissue and the liver in the present study. Male PRL-tg and control mice were castrated and placebo or testosterone (T7.5 or T30) pellets were implanted. Earlier studies have demonstrated that testosterone affects hepatic PRLR expression. In male rodents, castration resulted in an increased number of liver PRL-binding sites, while testosterone administration resulted in a decrease [19,20]. In this study, PRL induced L-PRLR mRNA in both adipose tissue and the liver. However, serum androgen levels affected liver and adipose tissue PRLR expression differently. In agreement with earlier studies, elevated testosterone levels decreased the PRLR mRNA expression in the liver of both PRL-tg and control mice. In contrast, PRLR expression was increased in the adipose tissue of control mice with elevated testosterone levels implanted with a T30 pellet. Furthermore, the PRLR expression in the adipose tissue of both PRL-tg and control mice was unaffected by castration. These results demonstrate a tissue-specific regulation of PRLR expression in adipose tissue and the liver.

In summary, the present study demonstrates increased resistin mRNA expression in the epididymal adipose tissue of untreated PRL-tg male mice and in male mice with elevated serum testosterone levels. In addition, male mice with elevated testosterone levels also have increased PRLR expression in the adipose tissue. These results suggest testosterone to be a regulator of both resistin and PRLR expression in mouse adipose tissue. As resistin has been demonstrated to antagonize insulin's action in rodents, the hormonal regulation of resistin and PRLR in this study could represent a link between PRL, testosterone and altered glucose metabolism.

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