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Higher 11-β-hydroxysteroid dehydrogenase type I gene expression in white adipose tissue in male than female rats

Wyższa ekspresja genu dehydrogenazy 11-β-hydroksysteroidowej w białej tkance tłuszczowej u samców niż samic szczurów

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Abstract

Background: 11-β-hydroxysteroid dehydrogenase type I (11βHSD1) in the white adipose tissue (WAT) of rats catalyses the conversion of 11-dehydrocorticosterone to corticosterone, a more active glucocorticosteroid. Glucocorticosteroids in WAT stimulate adipocytes differentiation and increase adipocytes size. The aim of this study was to examine the association between expression of 11βHSD1 in the WAT of male and female rats and adipose tissue mass as well as body mass.
Material and methods: Perirenal WAT from male and female Wistar rats aged three months, and ovariectomized females of the same age, was used in the study. 11βHSD1 gene expression was assayed in the perirenal WAT of rats by real-time PCR.
Results: 11βHSD1 gene expression in the perirenal WAT of male rats was higher than in female rats. The WAT and body mass of male rats was also higher than in females. 11βHSD1 gene expression in the perirenal WAT as well as WAT mass and body mass increased simultaneously after ovariectomy.
Conclusions: The results presented in this paper suggest that higher 11βHSD1 gene expression in the WAT is associated with higher body and adipose tissue mass. Moreover, our results suggest that oestradiol can modulate 11βHSD1 gene expression in the WAT of rats.
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Key words: *11-β-hydroxysteroid dehydrogenase type I, corticosterone, adipose tissue mass*

Streszczenie

Wstęp: Dehydrogenaza 11-β-hydroksysteroidowa typu 1 (11βHSD1) w tkance tłuszczowej szczurów katalizuje przemianę mniej aktywnego 11-dehydrokortykosteronu w bardziej aktywny kortykosteron. Glukokortykosteroidy stymulują różnicowanie adipocytów i powodują zwiększenie ich rozmiarów. Celem pracy było zbadanie zależności pomiędzy ekspresją 11βHSD1 w tkance tłuszczowej z okolicy nerek samców i samic szczura a masą tkanki tłuszczowej oraz masą ciała.
Materiał i metody: W białej tkance tłuszczowej z okolicy nerek pobranej od samców i samic oraz samic poddanych owariektomii szczurów rasy Wistar w wieku 3 miesięcy badano poziom mRNA 11βHSD1 metodą *real-time* PCR.
Wyniki: U samców stwierdzono znacząco wyższą ekspresję genu 11βHSD1 w tkance tłuszczowej niż u samic. Samce szczurów miały również wyższą masę ciała i masę tkanki tłuszczowej niż samice. Owariektomia spowodowała równoczesne zwiększenie masy ciała, masy tkanki tłuszczowej oraz wzrost ekspresji genu 11βHSD1 w tkance tłuszczowej z okolicy nerek u samic szczurów.
Wnioski: Przedstawione wyniki wskazują, że wyższa ekspresja genu 11βHSD1 wiąże się z większą masą ciała oraz tkanki tłuszczowej. Ponadto przedstawione wyniki wskazują, że ekspresja genu 11βHSD1 w tkance tłuszczowej zależy od estradiolu.
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Słowa kluczowe: *dehydrogenaza 11-β-hydroksysteroidowa typu I, kortykosteron, masa tkanki tłuszczowej*

Introduction

11-β-hydroxysteroid dehydrogenase type I (11βHSD1) in rats catalyses conversion of less active 11-dehydrocorticosterone to more active corticosterone in the liver, white adipose tissue (WAT), brain and other tissues. This reaction leads to local, intracellular, tissue-specific increase of corticosterone concentration [1]. Glucocorticoids are important in regulating WAT metabolism. In rat adipocytes, corticosterone promotes differentiation and inhibits preadipocytes proliferation, leading to increases of the sizes of these cells and adipose tissue mass [1]. It has been postulated that increased activation of glucocorticosteroids in the adipose tissue of humans plays an important role in the development of central obesity and metabolic syndrome [2], that may result in several comorbidities including cardiovascular disease and depression [3, 4].

Mice with overexpression of 11βHSD1 gene display enhanced adipocytes differentiation, and have higher

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adipose tissue mass [5]. 11βHSD1 gene expression has also been found to increase in the WAT of obese Zucker rats [6] and in the adipose tissue of morbidly obese patients [7]. It has been shown that the mass of adipose tissue is associated with the serum adiponectin and leptin concentrations [8, 9]. Male rats of the same age are bigger than females, and possess higher adipose tissue mass. The results of studies by Andersson et al. [10] and Paulsen et al. [11] suggest that oestrogen could influence 11βHSD1 gene expression in WAT. Collectively, the above presented data suggests that 11βHSD1 gene expression in the WAT of male and female rats could be associated with body and fat mass and is regulated by sex hormones.

The aim of the study was to test this hypothesis.

Material and methods

Animals

Eight male and eight female Wistar rats (*Rattus norvegicus*) of the same age (three months) were housed in wire-mesh cages at 22°C under a 12h light/12h dark cycle with lights on at 7:00 a.m. Food and water were provided *ad libitum*. The rats were killed (between 8:00 and 10:00 a.m.) by cervical dislocation under ketamine anaesthesia, and their WAT was weighed, and rapidly frozen in liquid nitrogen. The tissues were stored at –80°C until analysis. There were differences in the WAT distribution between the male and the female rats. For example, there was a large epididymal depot of WAT in males and a periovarian depot of WAT in females. We decided to study 11βHSD1 gene expression in perirenal WAT — an adipose tissue depot found both in males and females.

Ovariectomy of female rats

Eight two month-old female rats underwent bilateral ovariectomy. The surgery was performed under ketamine anaesthesia as described previously [12]. Eight sham-operated female rats served as controls in this experiment. For 30 days after the operation, the rats were housed under the same conditions as described above.

Gene expression analysis

Total cellular RNA was extracted from frozen WAT by isothiocyanate — phenol/chloroform method. cDNA was synthesised from 1 μg of total RNA using RevertAidTM First Strand cDNA Synthesis Kit (Fermentas, Canada). Each RNA sample was treated with RNase-free DNase I (Fermentas) before cDNA synthesis. 11βHSD1 mRNA levels in perirenal WAT were analysed by real-time PCR in iCycler iQ Real Time Detection System (Bio-Rad). The reaction was performed using iQ SYBR Green Supermix (Bio-Rad). β-actine gene was used as a standard.

The primers sequences were: F 5`-AATGCTCCAGGGGAAGAAAG, R 5`-AGTTCAAGGCAGCGAGACAC for 11βHSD1 and 5′-TGTCACCAACTGGGACGATA , R 5′-GGGGTGTTGAAGGTCTCAAA for β-actin. Amplification of specific transcripts was confirmed by obtaining the melting curve profiles and agarose gel electrophoresis of amplification products. Statistical analysis was performed using Microsoft Excel. The statistical significance of the differences between parameters studied was assessed by two-tailed t test.

Results

11βHSD1 mRNA level measured in the perirenal WAT was more than twice as high in males than in females (Figure 1A). Perirenal adipose tissue mass was significantly lower in females than in males (Figure 1B). Body mass of female rats was also significantly lower than the body mass of male rats of the same age (Figure 1C). The gender-related difference in 11βHSD1 gene expression in the WAT inclined us to examine the expression of this gene in the WAT of ovariectomized females. We found a significant increase of 11βHSD1 mRNA level after ovariectomy (Figure 2A). We also observed a significant increase of perirenal WAT mass (Figure 2B) as well as body mass (Figure 2C) in female rats after ovariectomy.

Discussion

In this paper, we present for the first time evidence that 11βHSD1 gene expression in the perirenal WAT of rats is higher in males than females (Figure 1A). Higher 11βHSD1 gene expression in subcutaneous adipose tissue has already been reported in lean men compared to lean women [13]. In rats, the gender-related differences in 11βHSD1 have been studied only in the liver. Low et al. [14] showed higher 11βHSD1 activity in the livers of male than female rats.

11βHSD1 gene expression is higher in the adipose tissue of obese rats and humans [5, 6].

Our results corroborate with the above, because we observed an association between 11βHSD1 mRNA level in WAT and adipose tissue mass as well as body mass in rats. Female rats had lower body mass and perirenal WAT mass than males (Figure 1B, C). Lower 11βHSD1 gene expression in the adipocytes of female rats would cause lower intracellular dehydrocorticosterone concentration in the WAT. That would be associated with lower level of adipocytes differentiation, and consequently lower adipose tissue mass of females compared to males.

Another factor responsible for the gender-related difference in 11βHSD1 gene expression in WAT could be 17β-oestradiol. Our results showed that ovariectomy led to an increase of 11βHSD1 gene expression

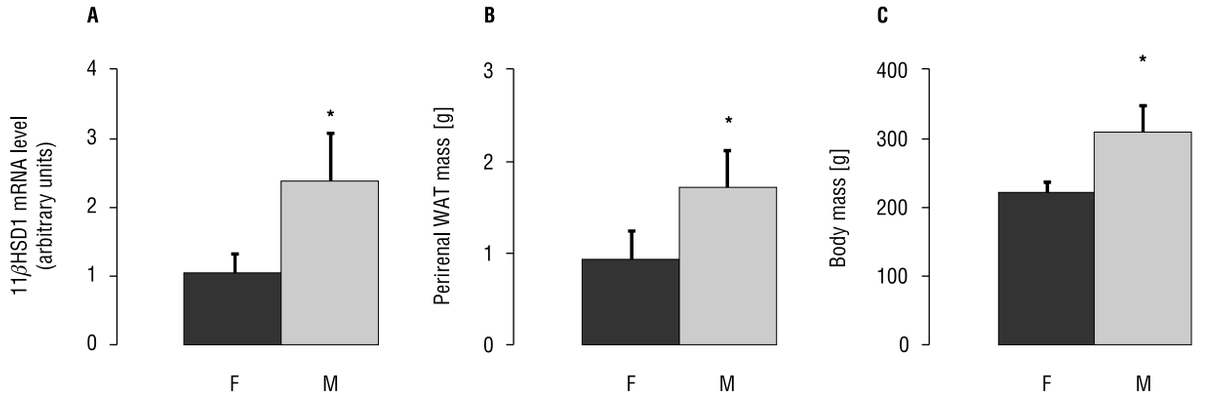

Figure 1. *11βHSD1 mRNA level in perirenal WAT (A), perirenal WAT mass (B) and body mass (C) of female rats (F) and male rats (M) (*p < 0.01)*

Rycina 1. *Poziom mRNA 11βHSD1 w tkance tłuszczowej z okolicy nerek (A), masa okołonerkowej tkanki tłuszczowej (B) oraz masa ciała (C) samic (F) i samców szczurów (M) (*p < 0,01)*

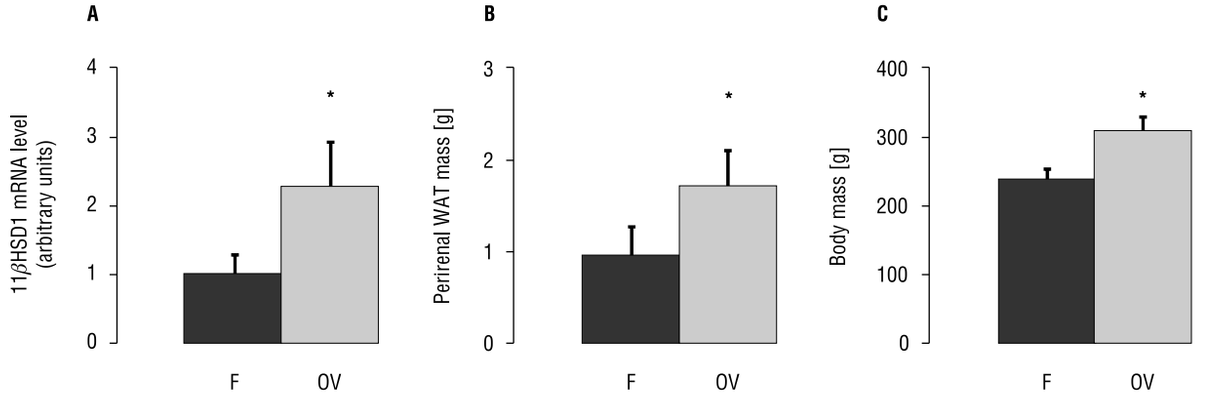

Figure 2: *11βHSD1 mRNA level in perirenal WAT (A), perirenal WAT mass (B) and body mass (C) of control female rats (F) and ovariectomized female rats (OV) (*p < 0.01)*

Rycina 2: *Poziom mRNA 11βHSD1 w tkance tłuszczowej z okolicy nerek (A), masa okołonerkowej tkanki tłuszczowej (B) oraz masa ciała (C) samic kontrolnych (F) i samic po owariektomii (M) (*p < 0,01)*

in perirenal WAT (Figure 2A) to the level similar to that observed in male rats (Figures 1A, 2A). Thus, our paper confirms the effect of ovariectomy on 11βHSD1 gene expression in WAT presented recently by Paulsen et al [11]. Andersson et al. reported that administration of oestradiol to ovariectomized rats reduced 11βHSD1 activity and mRNA level in liver and visceral WAT [10]. Our results, together with those reported by Andersson and Paulsen [10, 11], suggest an inhibitory effect of oestradiol on 11βHSD1 gene expression. Moreover, after ovariectomy, the perirenal WAT mass increased to a level similar to that observed in male rats (Figures 1B, 2B). Considering these results, one can suppose that a decrease of the 17β-oestradiol level after ovariectomy leads to increased corticosterone concentration in the WAT of rats, which in turn increases WAT and body mass. In conclusion, our study shows for the first time higher 11βHSD1 gene expression in the perirenal WAT of male than female rats. The gender-related difference in 11βHSD1 gene expression in WAT is associated with adipose tissue mass and oestradiol secretion.

Acknowledgements

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