



Local metabolism of glucocorticoids in Prague hereditary hypertriglyceridemic rats – Effect of hypertriglyceridemia and gender

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ABSTRACT

11 β -Hydroxysteroid dehydrogenase type 1 (11HSD1) is a microsomal NADPH-dependent oxidoreductase which elevates intracellular concentrations of active glucocorticoids. Data obtained from mouse strains with genetically manipulated 11HSD1 showed that local metabolism of glucocorticoids plays an important role in the development of metabolic syndrome. Tissue specific dysregulation of 11HSD1 was also found in other models of metabolic syndrome as well as in a number of clinical studies. Here, we studied local glucocorticoid action in the liver, subcutaneous adipose tissue (SAT) and skeletal muscles of male and female Prague hereditary hypertriglyceridemic rats (HHTg) and their normotriglyceridemic counterpart, the Wistar rats. 11HSD1 bioactivity was measured as a conversion of [³H]11-dehydrocorticosterone to [³H]corticosterone or vice versa. Additionally to express level of active 11HSD1 protein, enzyme activity was measured in tissue homogenates. mRNA abundance of 11HSD1, hexoso-6-phosphate dehydrogenase (H6PDH) and the glucocorticoid receptor (GR) was measured by real-time PCR. In comparison with normotriglyceridemic animals, female HHTg rats showed enhanced regeneration of glucocorticoids in the liver and the absence of any changes in SAT and skeletal muscle. In contrast to females, the glucocorticoid regeneration in males of HHTg rats was unchanged in liver, but stimulated in SAT and downregulated in muscle. Furthermore, SAT and skeletal muscle exhibited not only 11-reductase but also 11-oxidase catalyzed by 11HSD1. In females of both strains, 11-oxidase activity largely exceeded 11-reductase activity. No dramatic changes were found in the mRNA expression of H6PDH and GR. Our data provide evidence that the relationship between hypertriglyceridemia and glucocorticoid action is complex and gender specific.

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1. Introduction

Metabolic syndrome is defined as a cluster of disorders that includes abdominal obesity, dyslipidemia, hypertension, insulin resistance, and proinflammatory and prothrombotic states [1]. Similar symptoms can be found in patients with Cushing's syndrome, which is characterized by elevated plasma levels of glucocorticoids, and thus the disturbances of metabolic syndrome seem to be not only insulin-sensitive but also steroid-sensitive. However, since plasma levels of glucocorticoids are not raised in metabolic syndrome, the mechanism linking these two diseases was unknown until it was demonstrated that the glucocorticoid

responses in target tissues may depend not only on plasma levels of the hormone, but also on its local metabolism [2].

Local concentrations of glucocorticoid hormones are determined by 11 β -hydroxysteroid dehydrogenase (11HSD), a microsomal enzyme catalyzing interconversion of active (hydroxy-) forms of glucocorticoid hormones and their less active (keto-) derivatives. Two functionally and structurally different types of 11HSD have been described. Type 1 (11HSD1) acts predominantly as a NADPH-dependent reductase in intact cells and thereby increases local levels of active glucocorticoids in tissue. Its expression has been found in a wide variety of tissues, with the highest level in liver. Isoform 2 (11HSD2) is a NAD⁺-dependent oxidase that is expressed mainly in mineralocorticoid target tissues, such as the kidneys and colon. It metabolizes active glucocorticoids into less active derivatives and thus prevents it from binding to mineralocorticoid receptors [3].

11HSD1 has been extensively studied in relation to metabolic syndrome and obesity due to its high potential to increase

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intracellular level of biologically active glucocorticoids. It was found to be increased in the subcutaneous adipose tissue of obese individuals in a positive correlation with parameters of obesity and insulin resistance [4–9] but reduced in liver [4,10]. In contrast, studies of visceral adipose tissue provided rather inconsistent results showing either 11HSD1 upregulation [11,12] or no significant change [13–15]. In agreement with a higher susceptibility of men to developing visceral obesity, the whole body amplification of cortisol via 11HSD1 was shown to be higher in men compared to women [16,17].

The role of the local metabolism of glucocorticoids in the pathogenesis of metabolic syndrome is supported by the phenotypes of transgenic mice with genetically modified 11HSD1. Overexpression of 11HSD1 in adipose tissue resulted in visceral obesity together with glucose intolerance, dyslipidemia, hyperphagia, insulin resistance and hypertension [18,19]. With the exception of obesity, a similar phenotype was achieved in mice with liver-specific overexpression of 11HSD1 [20]. In contrast, 11HSD1 knockout mice displayed an opposite phenotype; they showed improved protection against hyperglycemia, dyslipidemia, deterioration of the lipoprotein profile and visceral fat accumulation [21–23]. Similar improvements in glucose tolerance, insulin sensitivity and lipid profile were achieved by pharmacological 11HSD1 inhibition [24–26].

Data from genetically modified animals suggest that glucocorticoid amplification in target tissues may contribute to insulin resistance, hypertension and dyslipidemia (hypertriglyceridemia, changes in lipoprotein and cholesterol profiles). However, the impact of 11HSD1 on lipemia under physiologically and/or more pathophysiologically relevant conditions has not been fully explored. To investigate the relationship between 11HSD1 and lipemia, we used Prague hereditary hypertriglyceridemic (HHTg) rats and the parental, normotriglyceridemic strain, Wistar rats. HHTg rats have been previously shown to be a non-obese model of metabolic syndrome [27] with the following symptoms: increased serum glucose, insulin, NEFA and triglycerides, alterations of insulin signaling pathways in key metabolic organs [28], and higher systolic, diastolic and mean arterial blood pressure [29] associated with hypertrophic heart and aorta [30]. To determine changes in 11HSD1 function during dyslipidemia, we examined hyper- and normotriglyceridemic rats for 11HSD1 activity and mRNA expression and for the glucocorticoid receptor (GR) mRNA expression in subcutaneous adipose tissue (SAT), liver and skeletal muscle, i.e., in tissues that determine lipemia. Since hexose-6-phosphate dehydrogenase (H6PDH) has been shown to directly influence the intensity and directionality of 11HSD1 activity [31,32], we also determined H6PDH mRNA levels. In addition, we examined sexual dimorphism in conjunction with 11HSD1 because this enzyme shows such a distinct dimorphism in rodent liver [33], and the females of JCR:LA corpulent rats show not only metabolic syndrome, but also greater hypertriglyceridemia than the males [34].

2. Materials and methods

2.1. Animals

Three-month-old male and female HHTg rats and Wistar rats (Institute of Physiology, Academy of Sciences of the Czech Republic, Prague) were housed in a room with controlled light cycles (12-h light/12-h dark) and temperature (22 °C), and with free access to standard laboratory chow and tap water. All procedures were approved by the Czech Academy of Sciences Animal Care and Use Committee and were carried out in accordance with the Guide for the Care and Use of Laboratory Animals, and the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

2.2. Measurement of 11HSD1 activity in tissue slices

11HSD1 reductase and oxidase activities were measured as a conversion of [³H]11-dehydrocorticosterone to [³H]corticosterone or vice versa, respectively. Fresh tissue slices (liver: 100 mg, SAT, skeletal muscle: 200 mg) were placed in 2 ml of serum-free RPMI saturated with pneumoxide into sealed vessels. Pneumoxide was used to provide sufficient oxygen level and stable pH in reaction during incubations. After a 10 min preincubation at 37 °C, radioactive substrate was added in a final concentration of 16 nmol l⁻¹ and the slices incubated for 60 min (liver) or 180 min (SAT, muscle). [³H]corticosterone was purchased from PerkinElmer (Waltham, MS, USA: specific activity 70 Ci mmol⁻¹) and [³H]11-dehydrocorticosterone was synthesized in-house from [³H]corticosterone using guinea pig renal microsomes. Reactions were stopped by rapid cooling on ice, and the steroids were extracted with Strata-X C18 cartridges and quantified by HPLC as described previously [35]. Tissue samples were dried and weighed. Ratio of oxidase to reductase activity was calculated for each sample.

2.3. Measurement of 11HSD1 activity in tissue homogenates

Fresh tissue samples were homogenized (1:9 w/v) in ice cold buffer containing 0.01 mol l⁻¹ Tris and 0.2 mol l⁻¹ sucrose (pH = 8.5) using a Polytron homogenizer (Kinematica AG, Littau, Switzerland). Homogenates were centrifuged at 400g and 4 °C for 10 min to remove cellular debris. SAT and muscle homogenates were used immediately to measure 11HSD1 activity. Liver homogenates were used for the isolation of the microsomal fraction as described previously [36] and 11HSD1 activity was measured in fresh microsomes. Protein contents were determined by the Bradford method [37].

11HSD1 activity was measured as a conversion of [³H]11-dehydrocorticosterone to [³H]corticosterone in incubation buffer containing 50 mmol l⁻¹ Tris, 100 mmol l⁻¹ KCl, 0.8 mmol l⁻¹ NADPH, 1 mmol l⁻¹ glucose-6-phosphate and 2 U glucose-6-phosphate dehydrogenase (pH = 8.5, 37 °C). After a 10 min preincubation [³H]11-dehydrocorticosterone was added in a final concentration of 16 nmol l⁻¹. The protein content and incubation times were 0.05 mg/reaction and 15 min for liver microsomes and 1 mg/reaction and 90 min for muscle and SAT, respectively. Reactions were stopped by rapid cooling on ice, and the steroids were extracted with Strata-X C18 cartridges and quantified by HPLC as described previously [35].

2.4. RNA extraction and gene expression analysis

Samples of for RNA expression analysis were snap-frozen and stored in liquid nitrogen. Hepatic and muscle RNA were isolated using RNA blue (TopBio, Prague, Czech Republic), and SAT RNA was isolated using the RNEasy Lipid Tissue Mini Kit (Qiagen, Inc.). RNA concentration and integrity was determined using a NanoDrop Spectrophotometer (NanoDrop Products, Wilmington, DE, USA) and an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Samples were treated with DNase, and 2 µg of RNA was used for reverse transcription with MMLV reverse transcriptase (Invitrogen, Inc.) and oligo dT (Roche, Mannheim, Germany).

The 11HSD1 mRNA level was quantified by real-time PCR with the QuantiTect SYBR Green PCR Kit (Qiagen, Inc.) on a LightCycler 2.0 (Roche, Mannheim, Germany) as described previously [36]. H6PDH and GR expression was quantified by real time PCR with LightCycler 480 SYBRgreen I Master mix on a LightCycler 480 (Roche, Mannheim, Germany). Standard curves for all genes were created by 10-fold dilution of mixed cDNA sample. Expression was normalized as described above. Primer sequences were: GR

forward AGGCCGGTCAGTGTTCCTAAT, reverse GCTGGGCAGTTTTCCTT. Primers for H6PDH were adopted from Balachandran et al. [38].

To identify the most stable housekeeping genes, a panel of genes was tested and the most suitable pair was identified by NormFinder for each tissue [39]. Hepatic and muscle 11HSD1 expression was normalized to a geometric mean of ATP synthase subunit 5B (ATP5B) and cytochrome c-1 (CYC1), whereas CYC1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used for SAT. Primer sequences were: ATP5B forward GCAGAAA-GAATCACCACCACCAA, reverse GCACGGGACAGCACAGTAGTAGCA, CYC1 forward GGCTCCTCCATCTACACAG, reverse ATCTTGAGTCC-CATGCGTTTTT, GAPDH forward AGCAATGCCTCTGCACCACCAAC, reverse CCGGAGGGGCCATCCACAGTCT. For housekeeping gene screening, the rat geNorm Housekeeping Selection Kit was purchased from PrimerDesign Ltd. (Southampton, UK).

2.5. Western blotting

Samples of liver, SAT and muscle were homogenized in ice cold buffer containing 0.25 mol l⁻¹ sucrose, 0.1 mol l⁻¹ Tris, 0.1 mmol l⁻¹ MgCl₂ and protease inhibitor cocktail (Roche, Mannheim, Germany). Microsomes were prepared as described above. Microsomal proteins (30 µg) were resolved in 10% polyacrylamide gel and transferred to ImmobilonP PVDF membrane (Millipore, Billerica, MA, USA). The membrane was blocked in PBS containing 0.05% Tween 20 (PBST) and 5% low fat milk and probed with goat anti-11HSD1 and rabbit anti-calnexin used as a normalization factor (1:1000 for 11HSD1, RD Systems, Minneapolis, MN, USA; 1:5000 for calnexin, Sigma-Aldrich, St. Louis, MO, USA) for 2 h at room temperature. Membrane was washed three times for 10 min in PBST followed by incubation with HRP conjugated secondary antibody (11HSD1:anti-goat, 1:25,000, Sigma-Aldrich, St. Louis, MO, USA; calnexin:anti-rabbit, 1:25,000, Pierce, Rockford, IL, USA) for 1 h at room temperature. The membrane was washed again and the signal was developed with Immobilon Western Chemiluminescent HRP substrate (Millipore, Billerica, MA, USA). Images were acquired with the use of LAS-1000 Image analyzer (FUJIFILM, Tokyo, Japan) and analyzed by AIDA evaluation software. Precision Protein StrepTactin-HRP Conjugate marker (Bio-Rad, Inc.) was used to determine molecular weights of protein bands. Despite the clear demonstration of 11HSD1 in blots of liver proteins we did not obtain positive staining in SAT and muscle proteins due to low sensitivity of the specific antibody and much lower expression of 11HSD1 in SAT and muscle in comparison with liver.

2.6. Serum analyses

Triglycerides (TG) were measured with a commercial kit obtained from Lachema (Brno, Czech Republic). HDL cholesterol (HDL), LDL + VLDL cholesterol (LDL/VLDL) and total cholesterol serum levels were measured with the EnzyChrom HDL and LDL/VLDL Assay Kit (BioAssay Systems, Hayward, CA, USA). Nonesterified fatty acids (NEFA) were measured with the NEFA C kit (Wako, Richmond, VA, USA). Corticosterone (CS) was determined using the commercial RIA kit (MP Biomedicals, Solon, OH, USA). All measurements were performed in triplicates.

2.7. Statistical analysis

All data are expressed as means ± SEM. 11HSD1 activity is shown as% of male Wistar rats activity which is considered to be 100%. Data were analyzed by two-way ANOVA to test strain and gender differences followed by post hoc Newman-Keuls test. Statistical analyses were done using the Statistica 6.1. package

(StatSoft, Inc., Tulsa, OK, USA) and a probability level of $p < 0.05$ was considered significant.

3. Results

As shown in Table 1, both male and female HHTg rats displayed increased serum levels of triglycerides in comparison with Wistar rats. No changes between the strains in serum levels of NEFA, HDL, LDL and total cholesterol were found in males or females, except for a slightly elevated LDL in HHTg males. Decreased serum levels of corticosterone were observed in female HHTg rats.

To investigate whether peripheral metabolism of glucocorticoids might contribute to dyslipidemia in HHTg rats, we examined distribution of 11HSD1 in metabolically active tissues in males and female or hypertriglyceridemic and normotriglyceridemic rats. As shown in Fig. 1, the 11-reductase activity of intact slices is tissue- and sex- and strain-specific. When compared to Wistar rats, the males of HHTg strain showed unchanged 11-reductase activity in liver but the activity was 2-fold increased in SAT and 2-fold decreased in skeletal muscle. In contrast to HHTg males, female HHTg rats displayed significantly higher hepatic 11-reductase activity than Wistar females while no differences were observed in SAT and skeletal muscle (Fig. 1). Similar pattern can be observed for 11HSD1 mRNA expression with the exception of the male SAT where the data are unreliable due to a large scatter and the female liver where 11HSD1 mRNA abundance was increased insignificantly (Fig. 2). Consistent with the findings of 11-reductase activity and 11HSD1 mRNA expression, the immunoblotting demonstrated strong 11HSD1 protein expression in males of both strains, much lower expression in HHTg females and nearly undetectable value in Wistar females (Fig. 3).

Next, we examined the presence of 11-oxidase activity in slices of the studied tissues. However, this activity was found only in SAT and skeletal muscle but not in liver. As demonstrated in Fig. 4, 11-oxidase activity did not show any sexual and strain difference in muscle but this activity was significantly higher in SAT of females in comparison with males of the same strain. In addition, female HHTg rats exhibited a significant reduction of 11-oxidase activity compared to female Wistar rats. We also calculated a ratio of 11-oxidase to 11-reductase activity to indicate the intracellular balance between corticosterone and 11-dehydrocorticosterone. In females, the inactivation of corticosterone predominates over corticosterone regeneration in both SAT and muscle. The ratio is significantly lower in females of HHTg rats, however not favorable

Table 1

Serum triglycerides (TG), total cholesterol (CHOL), HDL cholesterol (HDL), LDL cholesterol (LDL), nonesterified fatty acids (NEFA), corticosterone (CS), body weight in hypertriglyceridemic (HHTg) and normotriglyceridemic (Wistar) rats.

	Males		Females	
	Wistar	HHTg	Wistar	HHTg
TG (mmol l ⁻¹)	1.6 ± 0.19	2.33 ± 0.09***	1.14 ± 0.05†	2.15 ± 0.11***
CHOL (mg dl ⁻¹)	89.2 ± 3.9	93.3 ± 2.34	86.0 ± 7.42	77.1 ± 2.43
HDL (mg dl ⁻¹)	43.7 ± 2.44	40.0 ± 3.02	28.1 ± 3.46†	30.2 ± 3.85†
LDL (mg dl ⁻¹)	34.9 ± 2.64	47.9 ± 3.57*	56.6 ± 5.32***	55.5 ± 4.46
NEFA (mmol l ⁻¹)	0.36 ± 0.03	0.41 ± 0.05	0.41 ± 0.04	0.27 ± 0.02†
CS (ng ml ⁻¹)	458 ± 52	523 ± 57	723 ± 78†	510 ± 49*
Body weight (g)	301 ± 9	310 ± 15	210 ± 5	235 ± 6

Data are expressed as means ± SEM (n = 8–16).

* $p < 0.05$.

*** $p < 0.001$ Wistar vs. HHTg.

† $p < 0.05$.

*** $p < 0.001$ male vs. female.

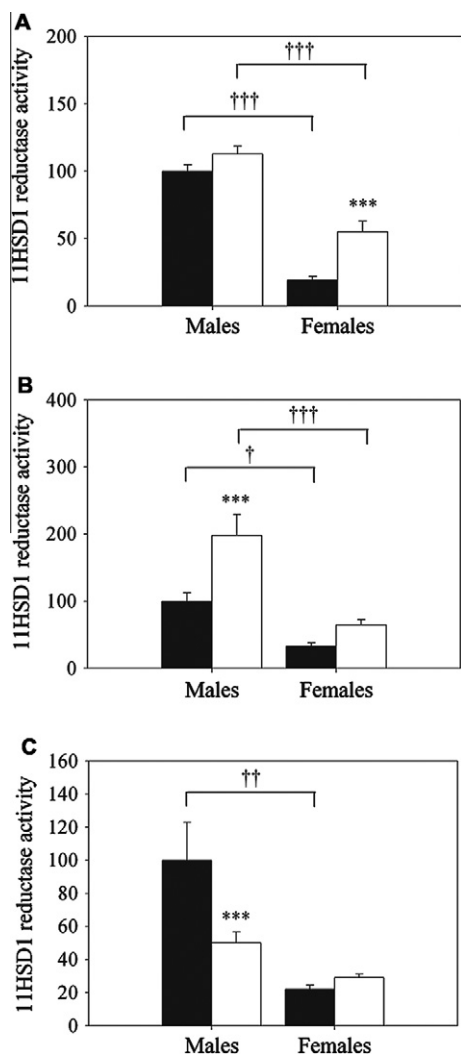


Fig. 1. 11-Reductase activity of 11HSD1 in tissue slices of the liver (A), subcutaneous adipose tissue (B) and skeletal muscle (C) of male and female hypertriglyceridemic (HHTg) (open bars) and normotriglyceridemic (Wistar) (full bars) rats. 11HSD1 activity is shown as% of male Wistar rats activity which is considered to be 100%. Data are expressed as means \pm SEM ($n = 8-10$). *** $p < 0.001$ Wistar vs. HHTg, † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$ male vs. female.

for 11-reductase activity. In contrast, males of both strains display almost equal levels of 11-oxidase and 11-reductase activity in SAT and muscle (Table 2).

To assess the level of active 11HSD1 protein we also measured 11HSD1 reductase activity in tissue homogenates in the presence of NADPH regenerating system. In contrast with findings in tissue slices, male HHTg rats exhibited a significant reduction of 11HSD1 activity not only in skeletal muscle but also in the liver and SAT (Table 3). In contrast with males, female HHTg rats displayed a significantly higher 11HSD1 activity in the homogenates of liver, SAT and skeletal muscle compared to Wistar females (Table 3). These striking discrepancies between 11-reductase activity in tissue slices and homogenates in SAT and muscle could be explained by different NADPH/NADP⁺ ratio in these measurements which is highly favorable for reductase activity in homogenates due to NADPH regeneration system.

To compare other determinants of glucocorticoid action in both phenotypes, we also examined the mRNA expression of GR and H6PDH (Table 4). Chronically increased regeneration of corticosterone in HHTg females was associated with an upregulation of GR

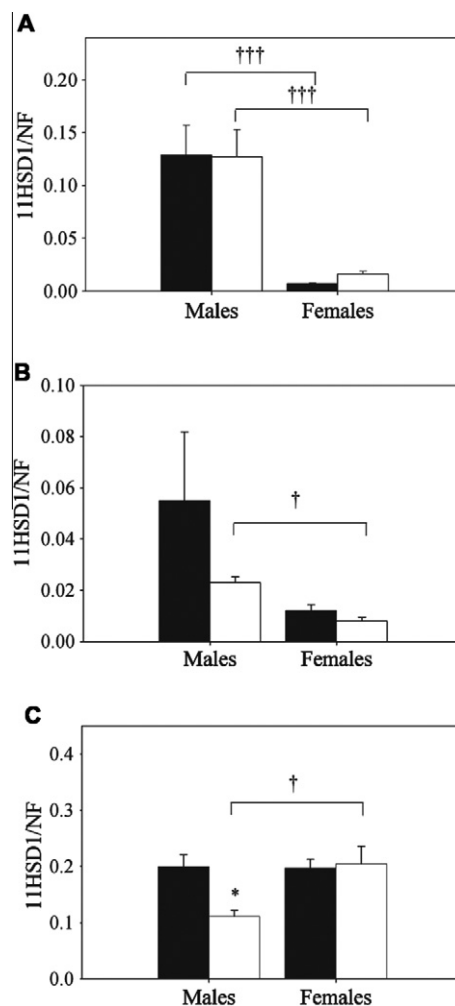


Fig. 2. 11 β -Hydroxysteroid dehydrogenase 1 (11HSD1) mRNA expression in the liver (A), subcutaneous adipose tissue (B) and skeletal muscle (C) of male and female hypertriglyceridemic (HHTg) (open bars) and normotriglyceridemic (Wistar) (full bars) rats. 11HSD1 mRNA level is expressed as a ratio of 11HSD1 concentration and normalization factor (NF, a geometric mean of the concentration of the two most stable housekeeping genes found in each tissue). For more details see Section 2. Data are expressed as means \pm SEM ($n = 8-16$). * $p < 0.05$ Wistar vs. HHTg, † $p < 0.05$ male vs. female, †† $p < 0.001$ male vs. female.

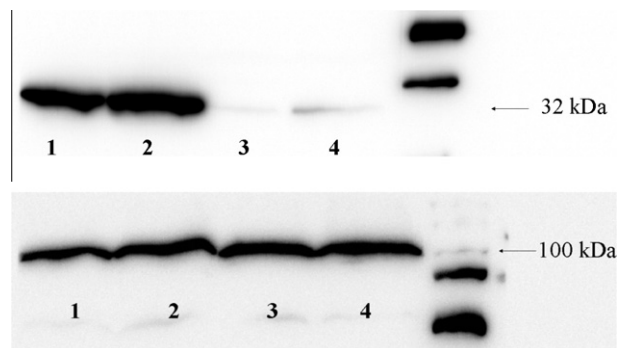


Fig. 3. Representative Western immunoblots images of 11 β -hydroxysteroid dehydrogenase 1 (11HSD1) and calnexin in the liver of male and female hypertriglyceridemic (HHTg) and normotriglyceridemic (Wistar) rats. (A) 11HSD1, (B) calnexin; (1) Wistar males; (2) HHTg males; (3) Wistar females; and (4) HHTg females.

expression in the liver. No significant changes were observed in SAT and skeletal muscle (Table 4). The decreased regeneration of

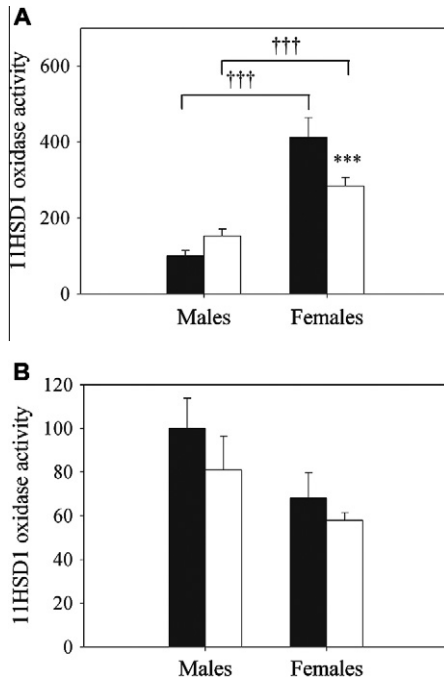


Fig. 4. 11-Oxidase activity of 11HSD in tissue slices of the subcutaneous adipose tissue (A) and skeletal muscle (B) of male and female hypertriglyceridemic (HHTg) (open bars) and normotriglyceridemic (Wistar) (full bars) rats. 11HSD1 activity is shown as % of male Wistar rats activity which is considered to be 100%. Data are expressed as means \pm SEM ($n = 8-10$). ** $p < 0.01$ Wistar vs. HHTg, *** $p < 0.001$ male vs. female.

Table 2

Ratio of 11-oxidase activity/11-reductase activity of 11HSD in tissue slices of the subcutaneous adipose tissue (SAT) and skeletal muscle of male and female hypertriglyceridemic (HHTg) and normotriglyceridemic (Wistar) rats. Ratio of oxidase to reductase activity was calculated for each sample.

	SAT		Muscle	
	Wistar	HHTg	Wistar	HHTg
Males	1.3 \pm 0.1	1.1 \pm 0.2	0.9 \pm 0.2	1.3 \pm 0.3
Females	19.8 \pm 6.5 ^{††}	6.6 \pm 1.0 [*]	2.2 \pm 0.3 ^{††}	1.4 \pm 0.1 [*]

Data are expressed as means \pm SEM ($n = 10$).

^{*} $p < 0.05$ Wistar vs. HHTg.

^{††} $p < 0.01$ male vs. female.

corticosterone observed in HHTg males was not accompanied by changes in GR mRNA either in the liver or SAT; small but significant upregulation was found in skeletal muscle. Strain-specific differences in 11HSD1 did not correlate with H6PDH expression, where no significant changes were observed.

Comparing males and females, we found distinct sexual dimorphism in 11HSD1 activity depending on the strain. In Wistar rats,

the females had significantly lower 11-reductase activity in tissue slices and homogenates in all examined tissues compared to males (Fig. 1, Table 3). Downregulation of 11-reductase activity was also found in the liver and SAT but not skeletal muscle of female HHTg rats (Fig. 1A and B). In contrast, 11-oxidase activity was significantly higher in females of both strains compared to males (Fig. 3). In both Wistar and HHTg rats, downregulation of 11HSD1 mRNA expression was observed in the liver and SAT of female rats (Fig. 2A and B), but the difference in SAT of HHTg rats was not significant. No difference between sexes was seen in skeletal muscle of Wistar rats, whereas 11HSD1 mRNA was upregulated in the muscle of HHTg females (Fig. 2C).

4. Discussion

Recent evidence suggests that liver- or fat-specific amplification of 11HSD1 might contribute to some metabolic complications, including obesity and metabolic syndrome. In addition, transgenic mouse models with aberrantly elevated 11HSD1 in adipose tissue or liver showed disorders typical for metabolic syndrome, including hypertension and deterioration of lipid profile [19,20]. Conversely, pharmacological inhibition of rodent 11HSD1 by specific blockers [25,26] or 11HSD1 knockout mice markedly improved the disorders associated with metabolic syndrome, including the metabolism of lipids [21]. However, it is unknown whether dysregulation of 11HSD1 in the liver or adipose tissue is associated with dyslipidemia under physiological and/or pathophysiological conditions.

In this study, we investigated the association between 11HSD1 and triglyceridemia using HHTg rats, which share the same genetic background as Wistar rats and have been previously shown to be a non-obese model of metabolic syndrome [27–30]. We demonstrated that hypertriglyceridemia found in HHTg rats is accompanied by gender-specific differences in local metabolism of glucocorticoids in the liver, SAT and skeletal muscle, i.e., in tissues that are responsible for plasma lipid levels. Enormous upregulation of hepatic 11HSD1 reductase activity in both tissue slices and homogenates in female HHTg rats compared to female Wistar rats resembles the phenotype of transgenic mice with selective overexpression of 11HSD1 in the liver (ApoE-11HSD1 mouse). Similarly to HHTg rats, this genetically manipulated mouse strain has been shown to develop dyslipidemia, hypertension and modest insulin resistance, but did not exhibit obesity and increased systemic levels of corticosterone [20]. This finding may indicate that the lipid phenotype present in HHTg rats may be attributable in part to glucocorticoid-induced changes in the liver, although, despite elevated glucocorticoid regeneration, systemic levels of corticosterone were slightly lower in female HHTg rats. However, HHTg males did not exhibit upregulation of 11HSD1, even if their serum concentration of triglycerides was higher than in Wistar males, and these gender differences could not be explained by other determinants of glucocorticoid action. First, chronically increased glucocorticoids have been shown to downregulate

Table 3

11HSD1 activity in tissue homogenates in liver, SAT and muscle.

	Liver		SAT		Muscle	
	Wistar	HHTg	Wistar	HHTg	Wistar	HHTg
Males	100 \pm 4.0	70 \pm 3.0 ^{***}	100 \pm 8.8	44 \pm 7.4 ^{***,†††}	100 \pm 15.6	9 \pm 1.6 ^{***}
Females	2 \pm 0.4 ^{†††}	42 \pm 3.9 ^{***,†††}	11 \pm 2.6	53 \pm 5.4 ^{**}	23 \pm 5.2 ^{†††}	90 \pm 12.7 ^{***,†††}

11HSD1 activity is shown as % of male Wistar rats activity which is considered to be 100%. Data are expressed as means \pm SEM ($n = 8-16$).

^{**} $p < 0.01$.

^{***} $p < 0.001$ Wistar vs. HHTg.

^{†††} $p < 0.001$ male vs. female.

Table 4

mRNA expression of the glucocorticoid receptor (GR) and hexose-6-phosphate dehydrogenase (H6PDH) in the liver, subcutaneous adipose tissue (SAT) and skeletal muscle of male and female hypertriglyceridemic (HHTg) and normotriglyceridemic (Wistar) rats.

	Liver		SAT		Muscle	
	Wistar	HHTg	Wistar	HHTg	Wistar	HHTg
<i>Males</i>						
GR	16 ± 8	33 ± 9	10 ± 3	16 ± 1	29 ± 4	45 ± 6 [†]
H6PDH	2 ± 0.5	3 ± 0.5	12 ± 2	20 ± 2	22 ± 3	26 ± 4
<i>Females</i>						
GR	34 ± 5	58 ± 6 [*]	19 ± 4	19 ± 2	27 ± 6	22 ± 5
H6PDH	4 ± 0.8	5 ± 1	13 ± 3	16 ± 3	11 ± 3	23 ± 6

mRNA level is expressed as a ratio of 11HSD1 concentration and normalization factor NF (a geometric mean of the concentration of the two most stable housekeeping genes found in each tissue) multiplied by 10^{-3} , for more details see Section 2. Data are expressed as means ± SEM ($n = 8-16$).

^{*} $p < 0.05$ Wistar vs. HHTg.

[†] $p < 0.05$ male vs. female.

GR expression [40], but the increased liver corticosterone regeneration in HHTg females was accompanied by higher GR expression than in Wistar rats that seem to exhibit much lower regeneration. A similar relationship between the upregulation of liver 11HSD1 and GR was observed in inbred lines of Fat but not Lean mice [41]. In contrast to females, the comparison of 11HSD1 reductase activity in tissue slices of HHTg and Wistar males does not indicate any changes in liver corticosterone regeneration accompanied with no changes in GR expression in the hypertriglyceridemic strain, although level of active 11HSD1 protein represented by activity in tissue homogenates was decreased in HHTg rats. Second, it is well known that 11HSD1 amplification of corticosterone regeneration is critically dependent on H6PDH [31,32], and significant increases in H6PDH expression without changes in 11HSD1 expression may result in a change of 11HSD1 activity from 11-oxidase to 11-reductase [42]. However, our results did not indicate any difference between H6PDH expression in HHTg and Wistar rats. Also, no hepatic dehydrogenase activity was detected in any of our experimental groups. Emerging new evidence indicates in humans the gender specific interplay between serum levels of leptin and hepatic 11HSD1. 11HSD1 activity in liver negatively correlated with leptin in men whereas positively in women [43]. Similar observations were described in leptin-deficient females of ob/ob mice where leptin treatment markedly increased 11HSD1 activity and expression in liver [44]. Although estradiol attenuates expression of 11HSD1 [45], it has also been shown to increase the sensitivity to leptin in rat hypothalamus [46]. Consistent with these findings, the sexual dimorphism in hepatic 11HSD1 activity of HHTg rats might be explained by gender specific actions of estrogen and leptin. Furthermore, adrenal glands in females are much larger and display enhanced steroidogenesis compared to males. This is probably due to stimulatory effect of estrogens on adenohypophyseal secretion of ACTH [47,48]. Moreover, adrenal hypertrophy associated with increased serum corticosterone and triglycerides was also observed in obese female but not male SHR/N-corpulent rats [49]. In the work presented here, we have found sexual dimorphism in serum corticosterone in Wistar but not HHTg rats therefore we can assume that in our rat model metabolic syndrome is not associated with enhanced adrenal action.

A previous study established that a selective overexpression of 11HSD1 in adipose tissue (aP2-11HSD1 mouse) caused obesity, insulin-resistant diabetes, dyslipidemia and hypertension [18,19]. The HHTg model exhibits some phenotypic features similar to aP2-11HSD1 mice, we therefore studied the metabolism of glucocorticoids in SAT as well. HHTg males showed upregulation of 11-reductase 11HSD1 activity, however level of 11-oxidase activity was similar. In Wistar rats, 11-oxidase activity was even slightly higher than reductase activity, we can therefore assume that net

result of 11HSD1 action in SAT of males does not favor reactivation of corticosterone. Similar observations were found in males of some other rodent models of metabolic syndrome and obesity, such as Zucker fa/fa rats [50]. In females, 11HSD1 oxidase activity in SAT largely exceeded reductase activity in both strains. These findings indicate, that females might be protected from excessive glucocorticoid level in adipocytes not only by the low expression of 11HSD1 mRNA but also by predominating oxidative direction of the reaction catalyzed by 11HSD1. 11-Oxidase activity was also detected in cultured human SAT preadipocytes, where it switched to 11-reductase activity upon differentiation [51].

Comparison of skeletal muscles, the third tissue that largely determines the levels of circulating lipids, between HHTg and Wistar strains showed a reduced regeneration of corticosterone in males of HHTg rats even if such difference was not found between HHTg and Wistar. Several lines of evidence support the role of muscle 11HSD1 in a setting of metabolic syndrome and type 2 diabetes. Skeletal muscle 11HSD1 mRNA and protein upregulation have been reported in the streptozotocin-induced rat model of type 2 diabetes mellitus [52]. A positive correlation between skeletal muscle 11HSD1 and GR α mRNA expression and BMI, blood pressure and insulin resistance were found in humans [53]. This view is further supported by recent human and rodent studies showing that selective pharmacological inhibition or siRNA-mediated reduction of 11HSD1 decreases mRNA expression of enzymes of lipid metabolism and fatty acid oxidation [54,55]. It is therefore tempting to speculate that in HHTg rats muscle 11HSD1 does not seem to contribute to metabolic disturbances in this strain.

Comparison of 11HSD1 activities and 11HSD1 mRNA levels indicates that there is not a simple relationship between 11HSD1 activity and mRNA abundance. At present we do not have any explanation for the discrepancies between mRNA abundance and activity of 11HSD1. However, 11HSD1 activity has previously been demonstrated to be affected by homodimerization [56] and glycosylation [57] and similar lack of correlation between 11HSD1 transcription and activity in the liver, adipose tissue and skeletal muscle has also been found by others [58–63].

In summary, we characterized the peripheral metabolism of glucocorticoids in the liver, SAT and skeletal muscle of HHTg rats, a rodent model of non-obese metabolic syndrome associated with hypertriglyceridemia. In comparison with normotriglyceridemic Wistar rats that share the same genetic background with HHTg rats, female HHTg rats showed enhanced regeneration of glucocorticoids in the liver and no changes in SAT and muscle. In contrast, male HHTg rats showed unchanged glucocorticoid regeneration in liver, but upregulated regeneration in SAT and downregulated in muscle. Expression of GR and H6PDH, which determine glucocorticoid action together with 11HSD1, did not correlate with metabo-

lism of glucocorticoids and was mostly similar in both Wistar and HHTg rats. Thus the hypertriglyceridemic phenotype present in HHTg rats may not be attributable to glucocorticoid regenerative changes in the liver, SAT and skeletal muscle. However, our data provide evidence that the relationship between hypertriglyceridemia and local metabolism of glucocorticoids in the liver, SAT and skeletal muscle is complex and gender-specific.

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