Temporary defects in the plasma lipid and glucose homeostasis are frequent complications accompanying chronic treatment with 13-cis-retinoic acid (13cRA). White adipose tissue acts as an endocrine organ producing a variety of hormones (adipocytokines) including leptin, adiponectin, tumor-necrosis factor alpha (TNFα) and angiotensin II (Ang II), which influence lipid metabolism, systemic insulin sensitivity and inflammation. To study the effect of a short-term 13cRA administration on metabolism of epididymal fat tissue, we treated Wistar rats with five identical therapeutic doses of 13cRA (0.8 mg/kg b.w.) by gavage during a period of 10 days. Expression of adiponectin, leptin, TNFα and selected proteins such as adipocyte fatty acid binding protein (aP2), insulin-dependent glucose transporter GLUT4, peroxisome proliferator-activated receptor gamma (PPARγ) and retinoid X receptors (RXRs) was investigated using RT-PCR. Short-term treatment with therapeutic doses of 13cRA caused significant increase of the aP2, PPARγ and moderately RXRα gene expression. Similarly, the relative amount of mRNA for leptin and GLUT4 was increased, while the TNFα transcript was decreased after treatment with 13cRA. The gene expression and plasma concentration of adiponectin were without any significant changes. Since local adipose renin-angiotensin system (RAS) has been presumed to be involved in the regulation of fat tissue metabolism, we also investigated the gene expression of RAS components in epididymal fat depot. Our data has shown that 13cRA elevated Ang II receptor type 1 (AT1 receptor) - at both, mRNA and protein level. Thus, our results demonstrate that short-term 13cRA treatment is inducing alterations in fat tissue metabolism in relation to stimulated adipogenesis.

Key words: retinoic acid, nuclear receptor, adipose tissue, adipokines, renin-angiotensin system
INTRODUCTION

Retinoic acid, the carboxylic acid form of vitamin A, is formed by enzymatic oxidation of retinal, an oxidation product of retinol. It occurs in different isomers, all-trans-retinoic acid (atRA) and 9-cis retinoic acid (9cRA) are representing biologically active ligands for nuclear receptor superfamily, the retinoic acid receptors (RARα,-β,-γ) and the retinoid X receptors (RXRα,-β,-γ) (1). Another naturally occurring isomer is 13cRA. It is believed that it does not have any gene regulatory activity but it is thought to be a precursor for atRA and 9cRA, formed by 13cRA isomerization (2, 3). 9cRA is a biologically active ligand for both RARs and RXRs while atRA is capable to bind only to RARs (4). RXR binds to its specific DNA target sequences as a homodimer or as a heterodimer with RARs (RXR/RAR) and with other nuclear receptors comprising peroxisome proliferator-activated receptors (PPARs) forming PPAR/RXR heterodimer (4). Unlike RXR/RAR, which is activated by binding atRA or 9cRA to RAR moiety, PPAR/RXR heterodimer can transactivate transcription of target genes upon binding PPAR ligand (e.g. fatty acids or their derivatives) (5) and RXR ligand.

Adipose tissue is a major site of storage of vitamin A derivates (collectively named retinoids), and thus plays an active role in their homeostasis and metabolism (6). Moreover, adipose tissue is known to be a target organ for retinoic acid, where expression of retinoid and retinoid X receptors has been reported (7). PPARγ is a predominant form of PPARs in adipose tissue and its obligate binding partner is RXRα (5, 8). Retinoids affect at least two processes related to energy balance: adaptive thermogenesis and metabolism of adipose tissue with possible involvement in the control of adiposity, which offers a great potential for treatment of obesity and type II diabetes (6, 9). However, most of metabolic data regarding 13cRA effects were obtained after long lasting 13cRA therapy of acne in humans or in rats after treatment with relatively high pharmacological doses of the drug (10, 11, 12). To our knowledge, no one study focused on changes in adipose tissue in animals treated with relatively low therapeutic doses of 13cRA.

Since it was published than even a single dose of retinoic acid administration affects retinoic acid receptor expression after 24 h in rat (13) we investigated the effect of short-term treatment with 5 subsequent identical therapeutic doses of 13cRA on the adipose tissue metabolism in relation to gene expression of adipocytokines (leptin, adiponectin, TNFα), PPARγ and GLUT4 in the rat epididymal adipose tissue (EWAT). It was shown that these parameters of EWAT are rapidly change by fasting (14, 15, 16). Therefore we did not starve our animals before sacrificing them.

Presence of several components of the RAS in white adipose tissue has been demonstrated, suggesting a local Ang II-generating system (17, 18, 19). Ang II produced by adipose tissue may play a paracrine/autocrine role in the regulation of specific tissue function. Although the exact mechanism is unknown, Ang II has
been shown to act as a trophic factor in adipose tissue (20). Furthermore, Ang II may decrease adipose tissue blood flow, thus affecting adipose tissue metabolism (21) and may increase leptin production (22). Since it has been shown that retinoic acid inhibits local RAS in cell culture systems (23, 24), we also studied gene expression of RAS components - angiotensinogen, angiotensin-converting enzyme (ACE) and AT1 receptor in the EWAT after the short-term 13cRA therapy.

MATERIALS AND METHODS

Animals

Male Wistar rats (160 g; Dobra Voda, Slovakia) were housed at 23±2°C under a 12/12h light/dark cycle with free access to water and standard rat chow. After one week, animals were divided in two groups - control group and group, which was treated with 13cRA (Roaccutan®; 0.8 mg/kg body weight in tylose) by gavage. Rats were treated every second day by five subsequent identical doses of 13cRA during a period of 10 days. Control group of animals received only tylose. Rats were sacrificed by decapitation 24h after the last treatment. The animals were not starved before being sacrificed. Principles of laboratory animal care and all procedures were approved by the Animal Care Committee of the IEE SAS Bratislava, Slovak Republic. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health.

Total RNA isolation and semi-quantitative PCR

EWAT, representing total fat tissue (25), was excised, weighed, rapidly frozen in liquid nitrogen and stored at -70°C until assayed. Total RNA was isolated from 100 mg of frozen EWAT using Table 1. Primers and conditions used for RT-PCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>PCR product (bp)</th>
<th>Annealing temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT1</td>
<td>F: GCACAATCGCCATAATTATCC</td>
<td>444</td>
<td>54°C</td>
</tr>
<tr>
<td></td>
<td>R: CACCTATGTAAGATCGCTTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE</td>
<td>F: CCTGATCAACAAGGAGTTTGAGA</td>
<td>320</td>
<td>59°C</td>
</tr>
<tr>
<td></td>
<td>R: GCCAGCCTTCCAGGCAACACGAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiotensinogen</td>
<td>F: TTGGTGGAGAGCTTGGGCTCCCTCA</td>
<td>263</td>
<td>57°C</td>
</tr>
<tr>
<td></td>
<td>R: CAGACACTGAGGTTGCTGTTGCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin</td>
<td>F: CCTGGGCTTTGCTCTATCTG</td>
<td>244</td>
<td>61°C</td>
</tr>
<tr>
<td></td>
<td>R: AGCCAAGCTGGTGAGGATCGT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLUT4</td>
<td>F: TTTCCAGATGTTCTGCGGATG</td>
<td>220</td>
<td>60°C</td>
</tr>
<tr>
<td></td>
<td>R: TCAGTCATCTTCATCTGCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPARγ</td>
<td>F: CATTTCTGCTCCACACTATGAA</td>
<td>550</td>
<td>51°C</td>
</tr>
<tr>
<td></td>
<td>R: CGGGAAGGACTTTATGTATGAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin</td>
<td>F: CTCCACCACAAAGGGACTCTT</td>
<td>500</td>
<td>59°C</td>
</tr>
<tr>
<td></td>
<td>R: CTGGTCCACATTTTTTTTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aP2</td>
<td>F: AGCGTGAAGGGGACCTTTGT</td>
<td>185</td>
<td>55°C</td>
</tr>
<tr>
<td></td>
<td>R: ATGGTGGTGCACTTTCCCATC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFα</td>
<td>F: ACCACACGTGGTGTCTTTTG</td>
<td>256</td>
<td>58°C</td>
</tr>
<tr>
<td></td>
<td>R: TTAGCGGGCCAAGGCGAACCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renin</td>
<td>F: AGATCCACACCGGATAACATT</td>
<td>190</td>
<td>56°C</td>
</tr>
<tr>
<td></td>
<td>R: TCCCCTCAGATTGTGCCAGCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>F: AGATCCCAACCGGATAACATT</td>
<td>309</td>
<td>52°C</td>
</tr>
<tr>
<td></td>
<td>R: TCCCCTCAGATTGTGCCAGCA</td>
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</tbody>
</table>
RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, CA) according to the manufacturer’s directions. 2 µg of total RNA were reversely transcribed using Ready-To-Go You-Prime First-Strand Beads kit and pd(N), random hexamer primers (Amersham Biosciences, Buckinghamshire, GB). RNA and cDNA were prepared as described in Pinterova et al. (2000) and Zorad et al. (2006) (18, 26). PCR was performed by primers which are listed in Table 1. Polymerase chain reaction (PCR) amplification was performed in a total volume of 25 µl containing 250 µmol/l deoxyribonucleotides triphosphate, 1U DyNAzyme™ II DNA polymerase (Finnzymes, OY, Finland), 2 µl of first strand cDNA and 12.5 pmol of each primers. PCR reactions mixture was denaturated to 94°C/5 min and allowed to proceed for 35 cycles comprising denaturation 1 min at 94°C; annealing 1 min at specific temperature for each primer (Table 1); extension 1 min at 72°C and final extension was performed at 72°C for 7 min. The number of cycles was determined in order to be within linear range of amplification and it was also verified that the quantity of PCR products are directly proportional to the amount of cDNA used. The gene expressions of RARs and RXRs were performed at the conditions we reported previously (27). Specific PCR products were separated by electrophoresis in 2% agarose gels in the presence of ethidium bromide stain. The band intensities were quantified by optical densitometry using camera documentational system STS 62201 (Ultra-Lum, Inc., USA) and 1D Image Analysis software (Eastman Kodak) and normalized relatively to the corresponding constitutively expressed internal control gene GAPDH band intensity. Obtained values are expressing relative changes in specific gene expression in arbitrary units.

Plasma membrane preparation and Western blotting

Cell membranes from EWAT were isolated by rotor-stator homogenization in Tris-HCl buffer (10 mM Tris-HCl, 250 mM saccharose, 1 mM benzamidine, 1 mM phenylmethylsulphonyl fluoride; pH 7.4). Homogenates were centrifuged at 6000 x g for 7 min at 4°C. By further centrifugation of the supernatant at 14000 x g for 15 min at 4°C, the pellet of crude plasma membranes was obtained and the resuspended in Tris-HCl buffer (50 mM Tris-HCl; pH 7.6). Protein concentrations were measured using Lowry method with bovine serum albumin as a standard (28). Membranes were solubilized by adding buffer (1:1) containing 12.5 mM Tris-HCl, 6% sodium dodecyl sulfate, 15% glycerol, 75 mM dithiotreitol, 0.025% bromophenol blue; pH 7.0 and boiling at 100°C for 5 min.

For Western blotting, 40 µg solubilized fat tissue membranes were separated on 7.5% polyacrylamide gel electrophoresis and then electrotransferred to HYBOND membrane. After blocking, the membrane was incubated overnight at 4°C with rabbit IgG fraction of antibody (1:500) raised against a synthetic peptide corresponding to 14-23 amino acid sequence of AT1 receptor (29). After washing, the second antirabbit antibody linked to horseradish peroxidase (1:20000, Sevapharm, Praque, Czech Republic) was applied to the membrane. Protein bands containing immunoreactive AT1 receptor protein were visualized by exposing the membrane to enhanced chemiluminiscence reagent (Amersham Biosciences, Buckinghamshire, GB) according to the manufacturer's protocol and exposed to X-ray film. The band intensities were qualified by optical densitometry using camera documentational system STS 62201 (Ultra-Lum, Inc., USA) and 1D Image Analysis software (Eastman Kodak). The specificity of AT1 antiserum was determined by competition experiments with the peptide antigen (29, 30).

Assays

To determine the triglyceride content in adipose tissue, lipids were extracted by the Folch's method (31). The lipid extracts were dried under nitrogen and dissolved in isopropanol. The triglyceride concentration was measured by enzymatic method using a commercial kit (Roche, Basel,
Switzerland). Serum adiponectin, cholesterol and non-esterified fatty acids levels were measured by using commercial kits (RIA kit, Linco Research. St. Charles, USA) and (Randox, UK), respectively.

**Statistical analysis**

The results are expressed as the mean ± SEM. Statistical comparisons were assessed by t-test. Difference between groups was considered statistically significant at value p<0.05.

**RESULTS**

The body weight of rats was significantly decreased after 13-cis retinoic acid treatment but this was not accompanied by simultaneous decline in epididymal fat mass and therefore the adiposity index rather tended to increase (Table 2). The 13cRA treatment had no effect on serum postprandial level of cholesterol and free fatty acids as well as on triglyceride (TG) content in EWAT (Table 2). Surprisingly, we found reduced levels of serum TG after 13cRA treatment under our experimental conditions (Table 2).

**Table 2.** Selected metabolic and morphometric parameters of control and 13cRA-treated rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>13cRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>268±7</td>
<td>247±5*</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>2.11±0.18</td>
<td>1.79±0.13</td>
</tr>
<tr>
<td>Serum non-esterified fatty acids (mmol/l)</td>
<td>0.84±0.09</td>
<td>0.74±0.04</td>
</tr>
<tr>
<td>Serum TG (mmol/l)</td>
<td>2.25±0.22</td>
<td>1.5±0.12**</td>
</tr>
<tr>
<td>Adiposity index (%)</td>
<td>1.00±0.05</td>
<td>1.16±0.10</td>
</tr>
<tr>
<td>TG content in adipose tissue (mg/g tissue)</td>
<td>0.74±0.01</td>
<td>0.75±0.02</td>
</tr>
</tbody>
</table>

Control: control rats (n = 5), 13cRA: 13cRA treated animals (n = 7). Data are expressed as mean ± SE, *: p < 0.05 and ** p < 0.01 vs. controls.

![Fig. 1.](image) The gene expression of aP2 and PPARγ in epididymal fat depot of control rats (n = 5) and rats treated with 13cRA (n = 7), *: p<0.05. Data are expressed as mean ± SE.
After 13cRA treatment the gene expression of aP2, a typical marker of the late phase of preadipocyte differentiation, was significantly increased in epididymal fat depot and the level of the PPARγ mRNA was found to be substantially increased, as well (Fig. 1). The short-term treatment of rats with 13cRA affected the metabolism of the EWAT by elevating leptin and GLUT4 gene expression and, on the contrary, decreasing level of the TNFα transcript (Fig. 2). On the other hand, we have found both the gene expression of adiponectin and its serum level, without significant changes in 13cRA-treated rats (Fig. 3).

The gene expression of retinoid receptor subtype RXRα, the obligate binding partner for PPARγ, had a tendency to increase after 13cRA treatment without reaching statistical significance (Control: 0.87±0.16 vs. 13cRA: 1.06±0.15

![Graphical representation of gene expression changes with 13cRA treatment.](image)

Fig. 2. Effect of short-term 13cRA treatment on leptin, GLUT4 and TNFα gene expression in epididymal fat depot, *: p<0.05. Control: control rats (n = 5), 13cRA: 13cRA treated animals (n = 7). Data are expressed as mean ± SE.
[arbitrary units]). On the other hand, RARβ gene expression significantly decreased (Control: 0.34±0.08 vs. 13cRA: 0.21±0.06 [arbitrary units], p<0.05). Transcription of other retinoid receptor subtypes (RXRβ,-γ) and (RARα,-γ), respectively, was without significant changes (data not shown).

Fat tissue metabolism alteration induced by 13-cis retinoic acid had marked impact on the AT1 receptor expression, the level of its mRNA and plasma membrane protein were increased (Fig. 4). However, changes neither in angiotensinogen and ACE mRNA in EWAT nor in liver angiotensinogen mRNA

![Graph showing adiponectin serum level and adiponectin gene expression in epididymal fat tissue of control and 13cRA-treated rats.](image1)

**Fig. 3.** Adiponectin serum level and adiponectin gene expression in epididymal fat tissue of control and 13cRA-treated rats. Control: control rats (n = 5), 13cRA: 13cRA treated animals (n = 7). Data are expressed as mean ± SE.

![Graph showing effect of short-term 13cRA treatment on AT1 receptor gene and protein expression in epididymal fat depot.](image2)

**Fig. 4.** Effect of short-term 13cRA treatment on the AT1 receptor gene and protein expression in epididymal fat depot, *: p<0.05. Control: control rats (n = 5), 13cRA: 13cRA treated animals (n = 7). Data are expressed as mean ± SE.
level in 13cRA-treated animals were observed (Table 3). Renin mRNA signal was very weak not enabling quantification in both experimental groups.

DISCUSSION

Now it is established that 13cRA treatment via appetite suppression causes a lesser body weight gain (32, 33). In our experiment 13cRA-treated rats had lower body weight compared to controls but this was not accompanied with a decrease in epididymal adipose tissue mass and adiposity.

White adipose tissue plays an important role in the regulation of energy balance and acts as an endocrine organ (34, 35). We have clearly shown that PPARγ mRNA is significantly increased in 13cRA treated group compared to controls. Retinoic acids impact preadipocyte differentiation although their final effect depends on their concentration and isomer availability as well as on relative RAR and RXR availability in the cells (7). Contrary to high doses of retinoic acid which block via RARs the PPARγ and C/EBPα induction and inhibit adipogenesis, low doses are needed to provide sufficient 9cRA to ensure the activation of the RXR moiety of the PPARγ/RXR heterodimer (6) and thus, trigger adipogenesis. In fact, expression of RXR isoforms is up-regulated during adipocyte differentiation (7) and synthetic specific RXR ligands have been shown to promote adipogenesis (36). PPARγ induces transcription of many differentiation-dependent genes, including aP2, a marker of preadipocyte differentiation (37). Taking in account a lack of epididymal fat tissue mass reduction and increased levels of aP2 and PPARγ mRNA after short-term treatment with 13cRA in our experiment we suggest stimulated adipogenesis in epididymal fat tissue of rats treated with 13cRA.

The gene expression of RXRα, a partner for dimerization with PPARγ in adipose tissue, displayed moderate tendency to increase after 13cRA treatment. Although the increase did not reach statistical significance, RXR might favour forming of PPARγ/RXR heterodimer instead RAR/RXR, as evidenced by impaired RARβ isoform transcription. Retinoic acids have ability to automodulate the steady-state level of its RAR and RXR receptors, what seems to be an important feature of adipose tissue response to retinoic acids (6). We suggest that both members of PPARγ/RXR heterodimer can contribute to

<table>
<thead>
<tr>
<th></th>
<th>Control (arbitrary units)</th>
<th>13cRA (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver angiotensinogen mRNA</td>
<td>1.38±0.04</td>
<td>1.39±0.07</td>
</tr>
<tr>
<td>Epididymal angiotensinogen mRNA</td>
<td>0.69±0.11</td>
<td>0.83±0.10</td>
</tr>
<tr>
<td>Epididymal ACE mRNA</td>
<td>0.85±0.10</td>
<td>0.97±0.16</td>
</tr>
</tbody>
</table>

Control: control rats (n = 5), 13cRA: 13cRA treated animals (n = 7). Data are expressed as mean ± SE.
preadipocyte differentiation. We assume that during short-term treatment at given
dose of 13cRA its transcriptional active isomers 9cRA and/or atRA induced
retinoid-responsiveness in fat cells i.e. that balance between the isomers, as well
as the current RAR and RXR receptors availability in preadipocytes resulted in
increased rate of adipogenesis.

GLUT4 is not produced in preadipocytes but its expression is markedly
enhanced during the preadipocyte differentiation (37, 38). Furthermore, PPARγ
has been shown to play a significant role in activating GLUT4 transcription during
the terminal stages of adipogenesis (39). In vitro 9cRA, but not atRA, increased
adipocyte glucose uptake and it has been proposed that this effect might be due to
activation of the PPARγ/RXR heterodimer (40). We suggest that increased GLUT4
transcription is a consequence of stimulated adipogenesis.

Studies in vitro indicate that retinoids inhibit adipocyte leptin production (40,
41) and in vivo studies suggest that this effect is primary rather than as a
consequence of the adipose tissue mass reduction, usually observed after atRA
and vitamin A treatment (6, 42). However, Goiot et al. (43) have shown increased
leptin production by adipocytes incubated with atRA. In our study, 13cRA did not
reduce adipose tissue mass and we attribute the increased leptin expression in
13cRA-treated animals to augmented formation of new adipocytes.

Alterations in the lipid plasma profile such as fasting hypertriglyceridemia and
also glucose homeostasis dysregulation are frequent complications accompanying
the long lasting 13cRA therapy (44, 10). Despite of above we did not observe
significant difference in postprandial serum cholesterol and free fatty acid levels
between controls and 13cRA treated rats. Moreover, we found reduced serum TG
and not changed TG content in adipose tissue under our experimental conditions.
Retinoid-induced hypertriglyceridemia is resulting from impaired clearance of
triglyceride-rich particles which leads to hypertriglyceridemia-associated insulin
resistance (45). The 5-day-lasting treatment with 13cRA did not affect insulin
sensitivity (45), whereas chronic therapy (5 months) impaired insulin tolerance in
patients (44). From these findings it seems that the effect of 13cRA on lipid and
glucose homeostasis is depending on dosage and duration of the therapy.

Adiponectin, the adipose-specific hormone, is considered to be an important
marker of glucose homeostasis, since its serum concentration positively
 correlates with insulin sensitivity (46). Koistinen et al. (47) reported a
paradoxical increase in serum adiponectin level in spite of the 13cRA-induced
reduction in insulin sensitivity after 5-month-lasting therapy. Authors assumed
that the increase in the adiponectin, a potent enhancer of insulin sensitivity (48),
might be viewed as a physiological adaptation to counteract alterations in
impaired systemic glucose tolerance. PPARγ was reported to induce expression of
the adiponectin gene (49). Although the adiponectin gene expression was not
changed in our study, it is possible that the elevated PPARγ transcription would
increase adiponectin production if the 13cRA treatment prolongs.
In addition, administration of 13cRA inhibited gene expression of TNFα, a pro-inflammatory cytokine with ability to impair insulin action in adipose tissue in paracrine/autocrine manner (50). The TNFα is a potent negative inhibitor of adipogenesis and it prevents early induction of PPARγ (51, 52). We believe that short term administration of therapeutic doses of 13cRA inhibits TNFα production via activation of PPARγ/RXR heterodimer resulting in increased adipogenesis and elevated expression of PPARγ, GLUT4 and aP2 in adipose tissue.

There is growing evidence that the adipose Ang II regulates metabolism of the adipose tissue as well as the production of adipocytokines (21, 26). It has been shown that atRA regulates local RAS by decreasing Ang II synthesis and AT1 receptor gene expression (23, 24) and also that retinoids are potent inhibitors of the Ang II actions (24, 53). We have previously reported a weak decline in the AT1 receptor gene expression after 13cRA treatment in chemically induced rat mammary gland carcinoma (54). In this study, however, we did not find alterations in the relative amount of the adipose angiotensinogen and ACE mRNA, and also in the liver angiotensinogen transcript. Based on these results, we do not suppose altered formation of local adipose tissue Ang II. As for the AT1 receptor, we observed significant increase in receptor mRNA and protein in 13cRA-treated rats. The elevated AT1 receptors are probably associated with stimulated adipogenesis because preadipocytes have much less AT1 receptors than the adipocytes (55). From these data we conclude that in this model 13cRA administration did not inhibit local adipose RAS.

In conclusion, the results of our study clearly demonstrate the alterations in the EWAT metabolism during short-term 13cRA administration in rats. The increased aP2, PPARγ, GLUT4, leptin and decreased TNF-α gene transcription suggest stimulated formation of new adipocytes and thus improved insulin sensitivity of fat tissue. However, these early changes are opposite to impaired systemic insulin tolerance reported after long-term therapy. Explanation for our data is an attempt of adipose tissue to maintain lipid homeostasis and normal insulin action during early phase of 13cRA treatment. We assume that in humans, short-term therapy with therapeutic or sub-therapeutic doses of 13cRA might result in improvement of adipose tissue differentiation to increase its capacity to expand and prevent adipose tissue-related metabolic disturbances.

Acknowledgements: This study was supported by the grants VEGA 2/0162/08, 2/5090/25 and 2/0022/08 of the Grant Agency of Ministry of Education and Slovak Academy of Sciences (VEGA).

Conflicts of interest statement: None declared.

REFERENCES


43. Goiot H, Laigneau JP, Devaud H, Sobhani I, Bado A. Similarities and differences in the 
transcriptional regulation of the leptin gene promoter in gastric and adipose cells. FEBS Lett 

44. Koistinen HA, Remitz A, Gylling H, Miettinen TA, Koivisto VA, Ebeling P. Dyslipidemia and 
a reversible decrease in insulin sensitivity induced by therapy with 13-cis-retinoic acid. 

45. Stoll D, Binnert C, Mooser V, Tappy L. Short-term administration of isotretinoin elevates 
plasma triglyceride concentrations without affecting insulin sensitivity in healthy humans. 

46. Stefan N, Vozarova B, Funahashi T, et al. Plasma adiponectin concentration is associated with 
skeletal muscle insulin receptor tyrosine phosphorylation, and low plasma concentration 

47. Koistinen HA, Remitz A, Koivisto VA, Ebeling P. Paradoxical rise in serum adiponectin 
collection in the face of 13-cis-retinoic acid-induced insulin resistance. Diabetologia 2006; 
49: 383-386.

Biochimie 2004; 86, 779-784.

49. Iwaki M, Matsuda M, Maeda N, et al. Induction of adiponectin, a fat-derived antidiabetic and 

50. Hotamisligil GS. Inflammatory pathways and insulin action. Int J Obes Relat Metab Disord 

and reversal of adipocyte differentiation is accompanied by suppressed expression of PPARγ 

52. Craftorn WP, Heyd F, Hegyi K, Sethi JK. Tumor necrosis factor-α inhibits adipogenesis via a β-

53. Haxsen V, Adam-Stitah S, Ritz E, Wagner J. Retinoids inhibit the actions of angiotensin II on 

54. Tybitanclova K, Macejova D, Liska J, Brtko J, Zorad S. AT1 receptor and ACE mRNA are 
increased in chemically induced carcinoma of rat mammary gland. Mol Cell Endocrinol 2005; 
244: 42-46.

differentiation of human preadipocytes via angiotensin type 1 receptors. Diabetes 2002; 51: 
1699-1707.

Received: April 2, 2008
Accepted: November 6, 2008

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