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Effect of DHEA on endocrine functions of adipose tissue, the involvement of PPARγ

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Abstract

Dehydroepiandrosterone (DHEA), an adrenal steroid, is known to decrease body fat. Thus, it may also alter the endocrine functions of adipose tissue. The aim of this study was to determine if administration of DHEA might influence adiponectin gene expression and secretion from adipose tissue. We demonstrate here the inducing effect of exogenously administered DHEA on adiponectin gene expression in epididymal WAT and adiponectin levels in serum of rats fed a DHEA-containing diet (0.6%, w/w) for 2 weeks, accompanied by a reduction in epididymal adipose tissue mass. A corresponding increase in peroxisome proliferator-activated receptor γ (PPARγ) mRNA expression suggests that PPARγ may be involved in the up-regulation of adiponectin gene expression after DHEA treatment. The presented observations indicate that the positive effects of DHEA, which seems to play a protective role against insulin resistance and atherosclerosis, may be in fact indirect and due to up-regulation of adiponectin gene expression and stimulation of adiponectin secretion from adipose tissue.

Keywords: Adiponectin; Adiponectin receptor; Leptin; Dehydroepiandrosterone; PPARγ; Adipose tissue

1. Introduction

Dehydroepiandrosterone (DHEA), a hormone secreted almost exclusively by the cortex of the adrenal gland, is the major circulating steroid in humans. Nevertheless, its physiological role has not yet been fully clarified. Plasma levels of this hormone decrease progressively with age [1]. Associated with aging, the decline in plasma DHEA concentrations is linked to a number of disorders including obesity, insulin resistance, type 2 diabetes and atherosclerosis [2,3]. The molecular mechanisms underlying DHEA mode of action remain to be identified.

Numerous studies have shown that DHEA (or its metabolites) has fat-reducing properties and may function as an anti-obesity agent in various models of obesity [4–7]. DHEA administration inhibited fat accumulation and reduced weight gain in young rodents, whereas in adult rats it decreased body fat and body weight [4,6,8]. The fat-reducing effect of DHEA in lean rats was not mediated by a reduction in caloric intake [4,8]. In humans, DHEA therapy resulted in a substantial decrease in body fat [9].

Adipose tissue, in addition to its role as a specialised store of body fat, is now recognised to be an endocrine organ secreting several hormones, particularly adiponectin and leptin, and a variety of other protein factors that are involved in many aspects of organism physiology, including appetite control and metabolic responses to nutrients [10–13]. Changes in adipose tissue mass may influence the endocrine functions of this tissue, thus in our study we focused on the effect of DHEA as a fat-reducing hormone on adiponectin gene expression and secretion from rat white adipose tissue. Adiponectin, also known as Acrp30, apM1 and AdipoQ, is an adipocyte-derived plasma protein, which was identified in human and rodent adipose tissues [10–12]. Adiponectin levels in serum, in contrast to circulating leptin, which reflects the size of fat tissue, are inversely correlated with BMI [13–15]. This adipocyte-specific secretory protein seems to play an important role in the regulation of glucose and lipid metabolism and body weight [16,17]. Recombinant adiponectin increases insulin sensitivity and improves glucose tolerance in various animal models [16,17]. Furthermore, as was shown in numerous studies, adiponectin has anti-atherogenic properties [18–20]. Although adiponectin is expressed exclusively in adipose tissue, its expression and serum levels are reduced in humans and animals with obesity [11,14]. Moreover, weight reduction significantly elevated

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plasma adiponectin levels in humans [15]. As several investigations in rodents have shown that DHEA reduces the accumulation of fat, we aimed to determine if administration of DHEA might influence adiponectin gene expression and secretion by adipose tissue. Regulation of adiponectin gene expression remains to be elucidated. A recent study has shown that peroxisome proliferator-activated receptor γ (PPARγ), which is a well-known transcriptional activator of many adipocyte-specific genes, is required for adiponectin gene induction [21]. Moreover, a functional PPAR-responsive element (PPRE) in human adiponectin promoter has been identified [21]. DHEA has been characterised as a peroxisome proliferator [22]. This raised the possibility that DHEA supplementation to the diet could lead to a change in adiponectin gene expression in adipose tissue and serum adiponectin level. It has been shown in numerous studies that the administration of DHEA at a concentration of 6 g DHEA/kg diet (0.6%, w/w) ensures a good response in terms of a reduction in body weight gain and adipose tissue mass, the latter being of particular importance for adiponectin expression [7,8,23,24]. To determine the effect of DHEA on adiponectin synthesis and release from adipose tissue, an experiment was performed comparing adiponectin gene expression and secretion in rats fed standard rodent diet, with or without 0.6% DHEA.

2. Materials and methods

2.1. Animals and treatment

Male Wistar rats, weighing approximately 230 g at the start of the experiment, were housed individually in wire-mesh cages at 22 °C in an animal room with a 12-h light–dark cycle (lights on at 8:00 a.m.). The rats were randomly assigned to either the control group ($n = 6$) or the DHEA-treated group ($n = 6$). Rats from the control group were fed a rodent diet (1324N from Altromin, Lage, Germany; energy content 2.85 kcal/g); while those from the DHEA-treated group were fed the rodent diet to which DHEA was added at 0.6% (w/w). The animals were given ad libitum access to food and water. After 2 weeks, all the rats were killed by cervical dislocation between 8:00 and 10:00 a.m., and their trunk blood was collected. Epididymal adipose tissue and liver were taken from each experimental group. Tissue specimens were immediately frozen in liquid nitrogen and were stored at −80 °C until required for analysis. The experimental protocol was approved by the Medical University of Gdansk Ethics Committee for Animal Experimentation.

2.2. Determination of mRNA levels by the real-time RT-PCR

Total RNA was extracted from the frozen tissue specimens as previously described [25]. The amount of RNA obtained was determined by spectrophotometry at 260 nm; the A260/A280 ratios were always higher than 1.8. The integrity of RNA samples was checked on 1% agarose gels. Adiponectin, leptin, AdipoR1, AdipoR2, PPARγ, RXRα, ACO, LCPT-1, HSL, FAS and UCP-1 mRNA expression was quantified by the real-time RT-PCR using iCycler iQ Real Time Detection System (Bio-Rad, Hercules, USA). Primers were designed with Sequence Analysis software package (Informagen, Newington, USA) from gene sequences obtained from Ensembl Genome Browser (www.ensembl.org). Primer sequences are shown in Table 1.

One microgram of total RNA was reverse transcribed using AMV Reverse Transcriptase (Roche Molecular Biochemicals, Mannheim, Germany). Reactions were diluted to 100 μl, then 2 μl of each RT reaction was amplified in a 25 μl PCR mix containing 0.3 μM of each primer and iQ SYBR Green Supermix (Bio-Rad). Samples were incubated for an initial denaturation and polymerase activation at 95 °C for 5 min, followed by 40 PCR cycles each consisting of 95 °C for 20 s, 55 °C for 20 s, and 72 °C for 40 s. Analysis was performed with iCycler iQ software. Controls without RT and with no template cDNA were performed with each assay and all samples were run in triplicate. To compensate for variations in input RNA amounts, and efficiency of reverse transcription, β-actin mRNA was quantified and results were normalised to these values. Relative quantities of transcript were calculated using the $2^{-\Delta\Delta C_t}$ formula, where C_t is defined as the cycle number at which fluorescence is statistically significantly above background; ΔC_t is the difference in C_t of the gene of

Table 1
Primer sequences used for real-time RT-PCR

Gene	Primer sequences
Adiponectin	5′-TGGCAGAGATGGCACTCC-3′
NM_144744	5′-CTTCCGCTCCTGTCATTCC-3′
Leptin	5′-CTCATCAAGACCATTGTCACC-3′
NM_013076	5′-AGGTCTCGCAGGTTCTCC-3′
AdipoR1	5′-CCTCTCCATCGTCTGTGTCC-3′
NM_207587	5′-CACAGCCATGAGGAAGAACC-3′
AdipoR2	5′-CACAACCTTGCTTCATCTACC-3′
XM_232323	5′-TGAGCATTAGCCAGCCTATC-3′
PPARγ	5′-CTGTTATGGGTGAAACTCTGG-3′
AB019561	5′-GAAATCAACCGTGGTAAAGG-3′
RXRα	5′-CCTGATCGACAAGAGACAGC-3′
NM_012805	5′-CCACGTATGTCTCAGTCTTGG-3′
ACO	5′-TCCAGATAATTGGCACCTACG-3′
NM_017340	5′-GAGCTGAGCCAGAACTATTGC-3′
LCPT-1	5′-AAGAATGGCATCATCACTGG-3′
NM_031559	5′-CTCACAATGTTCTTCGTCTGG-3′
HSL	5′-CTCCTCATGGCTCAACTCC-3′
NM_012859	5′-ACTCCTGCGCATAGACTCC-3′
FAS	5′-AGCAGGCACACACAATGG-3′
NM_017332	5′-TCTCAGGATCTCTGCTCAGG-3′
UCP-1	5′-GCCAAGACAGAAGGATTGC-3′
NM_012682	5′-AGCCGAGATCTTGCTTCC-3′
β-actin	5′-TACAATGAGCTGCGTGTGG-3′
V01217	5′-TGGTGGTGAAGCTGTAGCC-3′

interest and C_t of β-actin; and $\Delta\Delta C_t$ is the difference in ΔC_t of unknown sample and ΔC_t of calibrator/control sample [26]. The results are expressed in arbitrary units, with one unit being the mean mRNA levels determined in the control group. Amplification of specific transcripts was further confirmed by obtaining melting curve profiles and subjecting the amplification products to agarose gel electrophoresis.

2.3. Adiponectin, leptin and insulin radioimmunoassay

Serum adiponectin, leptin and insulin levels were measured with rat-specific radioimmunoassay kits (Linco Research, St. Charles, USA). Assays were performed according to the manufacturer's instructions.

2.4. Determination of serum glucose and triacylglycerol levels

Glucose concentration in serum was determined by a spectrophotometric method using hexokinase and glucose 6-phosphate dehydrogenase (Alpha Diagnostics, Warszawa, Poland). Serum triacylglycerol levels were measured by an enzymatic method (Roche Diagnostics, Mannheim, Germany).

2.5. Statistical analysis

Statistical analysis was performed using Systat software (SPSS, Chicago, USA). The results, expressed in arbitrary units, are presented as means ± standard error of the mean (S.E.M.). The statistical significance of the differences between the DHEA group and control group was assessed by one-way analysis of variance (ANOVA) followed by Student's *t*-test or Mann–Whitney test. Correlation coefficients were determined by linear regression analysis. $P < 0.05$ was considered as a significant difference.

3. Results

3.1. Effect of DHEA on body weight and epididymal adipose tissue mass

Male Wistar rats were administered either standard rodent diet (control group) or diet supplemented with 0.6% DHEA for 14 days (DHEA-treated group). Initial body weight was approximately 230 g in both groups. After 2 weeks, rats fed the DHEA-containing diet weighed less than control ones (227 ± 12.1 g versus 273 ± 8.6 g, respectively; $P < 0.05$), although average food intake in

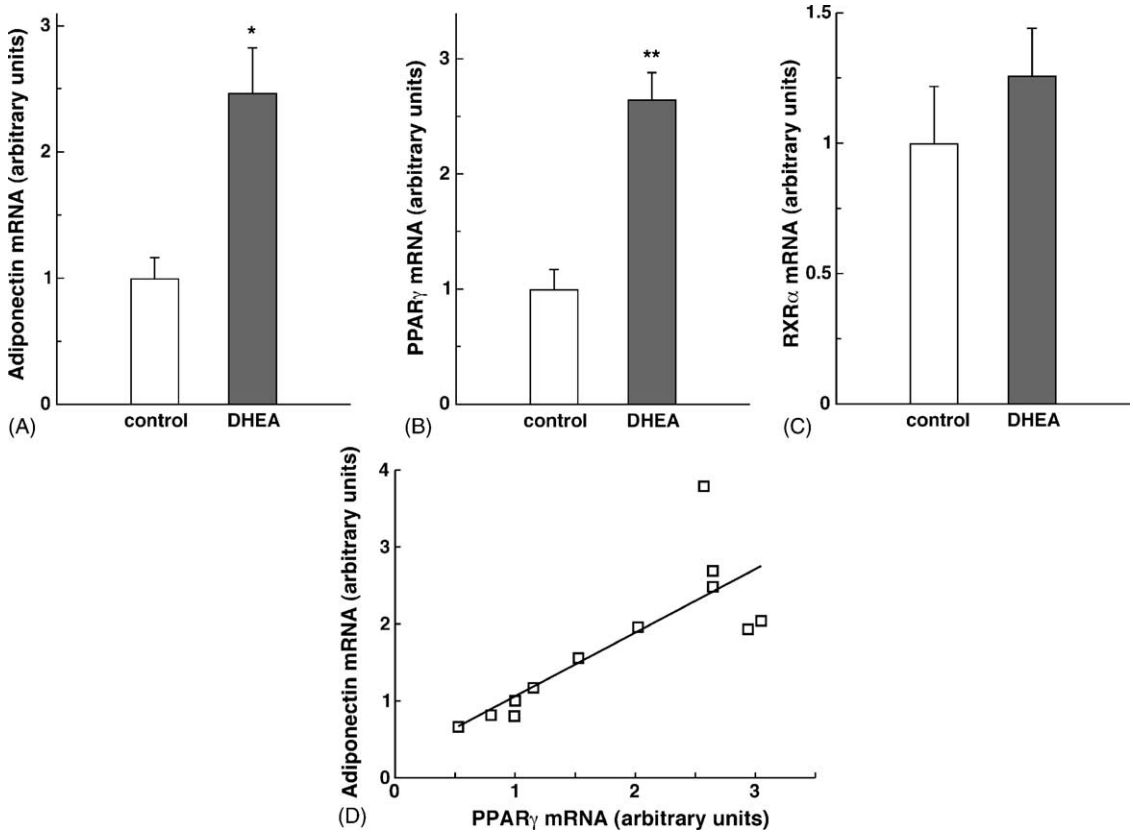

Fig. 1. Effect of DHEA on adiponectin (A), PPARγ (B) and RXRα (C) gene expression in white adipose tissue of control and DHEA-treated rats. Adiponectin, PPARγ and RXRα mRNA levels were measured with the real-time RT-PCR and normalised to β-actin using the $\Delta\Delta C_t$ method of relative quantification. Controls without RT and with no template cDNA were performed with each assay and all samples were run in triplicate. Data are reported as means ± S.E.M., $^{*}P < 0.05$, $^{**}P < 0.01$ compared to control. Adiponectin and PPARγ gene expression in white adipose tissue were positively correlated ($r = 0.814$, $P < 0.01$) (D).

both groups was not significantly different throughout the course of the study. Moreover, DHEA administration reduced epididymal adipose tissue mass from 3.47 ± 0.34 g in control rats to 2.63 ± 0.44 g ($P < 0.05$) in DHEA-treated rats. DHEA treatment did not affect circulating insulin and glucose levels (serum insulin: 0.17 ± 0.02 ng/ml versus 0.16 ± 0.03 ng/ml; serum glucose: 7.27 ± 0.42 mmol/l versus 7.36 ± 0.23 mmol/l in the control group versus DHEA-treated group, respectively), although there was a trend for triacylglycerols to be lower in rats fed DHEA-containing food as compared to control rats (135.2 ± 8.3 mg/dl versus 144.5 ± 11.2 mg/dl, respectively). To verify the response induced by DHEA treatment in rats, we quantified acyl-CoA oxidase (ACO) mRNA expression in livers of control and DHEA-treated rats. Acyl-CoA oxidase, the rate-limiting enzyme in the peroxisomal β-oxidation system, is a widely accepted marker of peroxisome proliferation [27], thus we chose this enzyme as an adequate control of DHEA action. ACO mRNA levels were determined by the real-time RT-PCR. DHEA administration resulted in a more than 16-fold increase in ACO mRNA expression in livers of DHEA-treated rats, as compared to controls (not shown).

3.2. DHEA increases adiponectin gene expression in adipose tissue and adiponectin levels in serum

The reduction in epididymal adipose tissue mass after DHEA treatment was accompanied by an increase in adiponectin gene expression. In adipose tissue of rats fed a diet supplemented with DHEA we observed a more than two-fold increase in adiponectin mRNA levels (Fig. 1A). Adiponectin is a secretory protein, thus radioimmunoassay was performed to determine serum adiponectin levels. As shown in Fig. 2A, DHEA administration elevated also adiponectin levels in serum (by 39%, as compared to control). Feeding rats the DHEA-containing diet had opposite effects on adiponectin and leptin gene expression in adipose tissue and led to a decrease in leptin mRNA levels in rat WAT [23]. Moreover, DHEA administration significantly lowered serum leptin levels (Fig. 2B). There was a negative correlation between leptin and adiponectin levels in serum ($r = -0.604$, $P < 0.05$) (Fig. 2C).

3.3. DHEA up-regulates PPARγ gene expression in adipose tissue

PPARγ has been proposed to be required for adiponectin gene induction in adipocytes [21]. Thus we aimed to determine whether up-regulation of adiponectin gene expression seen in epididymal adipose tissue of DHEA-treated rats is accompanied by an increase in PPARγ gene expression. In our study, treatment of rats with DHEA resulted in a significant (2.65-fold) increase in PPARγ mRNA levels in WAT (Fig. 1B). Moreover, RXRα (9-*cis*-retinoic acid receptor α) gene expression tended to be higher in adipose tissue of DHEA-treated rats (Fig. 1C). RXRα is involved in the formation of a heterodimer with PPARγ.

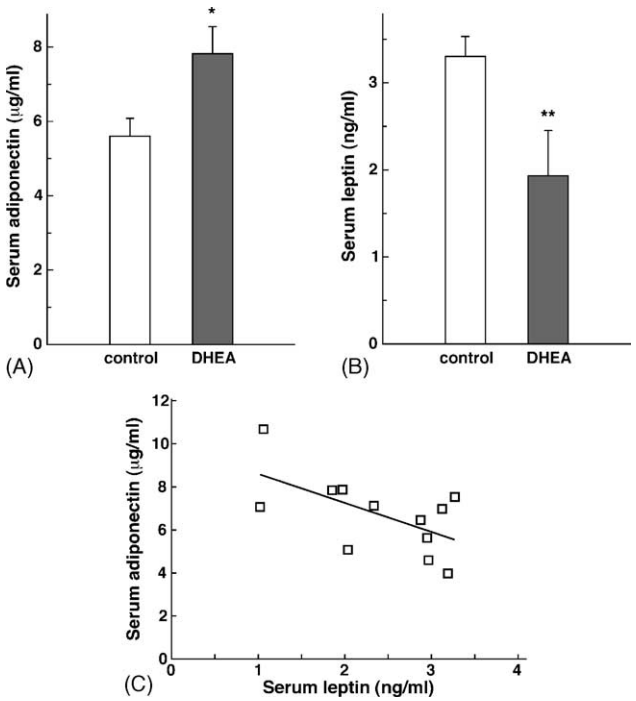

Fig. 2. Effect of DHEA on adiponectin (A) and leptin levels in serum (B) of control and DHEA-treated rats. Serum adiponectin and leptin levels were measured using rat-specific radioimmunoassay kit. Data are reported as means ± S.E.M., $^{*}P < 0.05$, $^{**}P < 0.01$ compared to control. Adiponectin and leptin levels in serum were negatively correlated ($r = -0.604$, $P < 0.05$) (C).

3.4. Effect of DHEA on adiponectin receptors, AdipoR1 and AdipoR2, gene expression

AdipoR1 and AdipoR2 gene expression was measured by the real-time RT-PCR in epididymal adipose tissue of rats from the control group and the DHEA-treated group. We observed a three-fold increase in AdipoR1 mRNA expression in rat WAT after DHEA treatment (Fig. 3A). However, DHEA had no effect on AdipoR2 mRNA levels in adipose tissue (Fig. 3B).

3.5. Effect of DHEA on LCPT-1, HSL, FAS and UCP-1 gene expression

Carnitine palmitoyltransferase 1 (LCPT-1), hormone-sensitive lipase (HSL) and fatty acid synthase (FAS) gene expression was determined by the real-time RT-PCR in epididymal adipose tissue of rats from the control group and rats fed the DHEA-containing diet. DHEA administration up-regulated the expression of the gene encoding LCPT-1, resulting in a 1.74-fold increase in LCPT-1 mRNA levels in WAT (Fig. 4A). Moreover, DHEA induced an approximately two-fold increase in HSL gene expres-

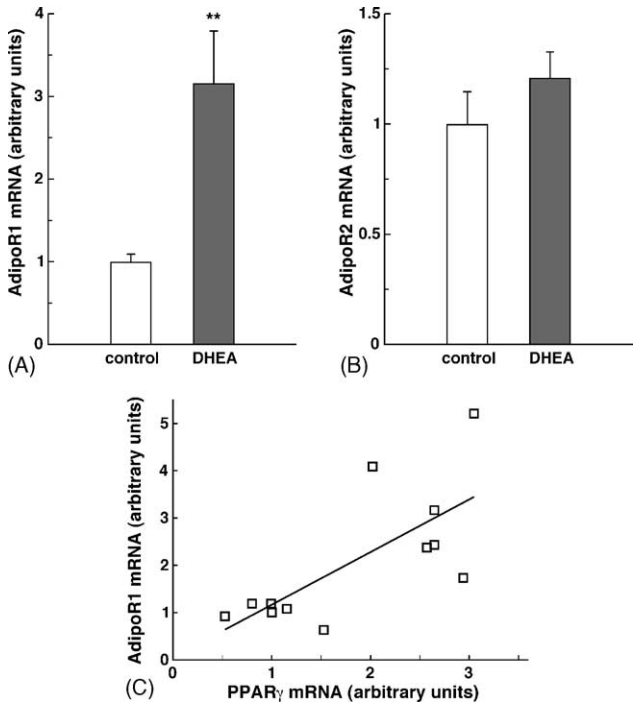

Fig. 3. Effect of DHEA on AdipoR1 (A) and AdipoR2 (B) gene expression in white adipose tissue of control rats and rats fed 0.6% DHEA-containing diet. AdipoR1 and AdipoR2 mRNA levels were measured with the real-time RT-PCR and normalised to β-actin using the $\Delta\Delta C_t$ method of relative quantification. Controls without RT and with no template cDNA were performed with each assay and all samples were run in triplicate. Data are reported as means ± S.E.M., $^{**}P < 0.01$ compared to control. AdipoR1 and PPARγ gene expression in white adipose tissue were positively correlated ($r = 0.717$, $P < 0.01$) (C).

sion in adipose tissue (Fig. 4B). It had no substantial effect on FAS gene expression in this tissue; however, FAS mRNA levels in WAT tended to be lower after DHEA treatment (Fig. 4C). Uncoupling protein 1 (UCP-1) gene expression was measured in brown adipose tissue. UCP-1 mRNA levels tended to be higher in DHEA-treated rats (we observed an increase in UCP-1 mRNA levels by 38%, $P > 0.05$; not shown).

4. Discussion

The aim of the present study was to investigate the effect of short-term DHEA administration on endocrine functions of rat adipose tissue. DHEA is the most abundant circulating steroid in humans; however its levels in the blood decline markedly with age [1]. The decline in DHEA concentrations is linked to a number of disorders, including obesity, insulin resistance, type 2 diabetes and atherosclerosis [2,3]. The physiological role of DHEA and the molecular mechanisms underlying its mode of action has not yet been fully elucidated. In contrast to humans, rats do not produce this adrenal steroid in significant amounts. However, a number of studies have shown that rat tissues are responsive to exogenously administered DHEA [6,8,23], therefore the rat is a useful model for studying the effects of this hormone. DHEA given to rodents elicits a pleiotropic response, which includes hepatomegaly and hepatic peroxisome proliferation associated with the induction of enzymes involved in the peroxisomal β-oxidation system and several other lipid-metabolising enzymes [22,28]. Peroxisomal acyl-CoA oxidase, the rate-limiting enzyme of the β-oxidation pathway in peroxisomes, is the most widely used marker of peroxisome proliferator action [22,27]. Thus, to verify the response of rats to DHEA administration we quantified ACO gene expression in livers of control and DHEA-treated rats. Feeding rats 0.6% DHEA-containing diet for 2 weeks resulted in a substantial (more than 16-fold) increase in ACO mRNA levels in liver, indicating the occurrence of peroxisome proliferation.

As shown in numerous studies, DHEA (or its metabolites) reduces the accumulation of fat [4–6,23]. When

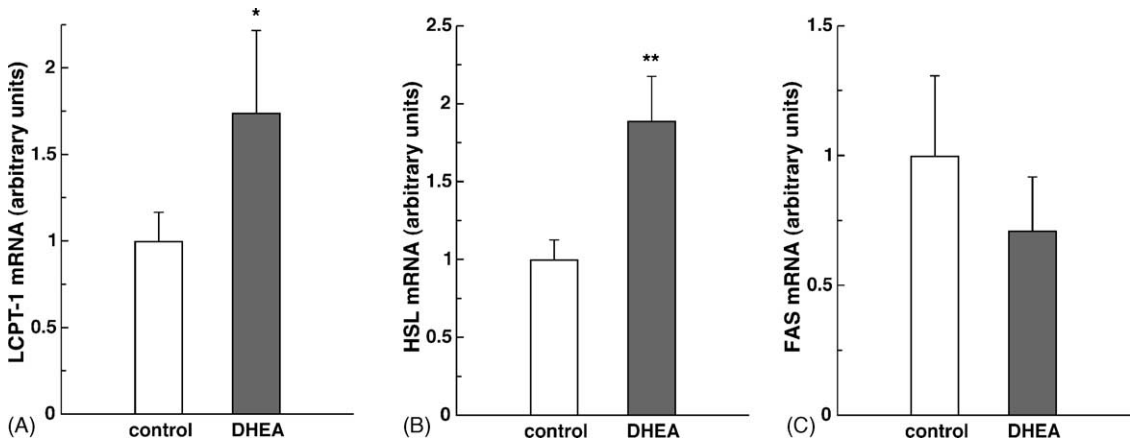

Fig. 4. Effect of DHEA on LCPT-1 (A), HSL (B) and FAS (C) gene expression in white adipose tissue of control and DHEA-treated rats. LCPT-1, HSL and FAS mRNA levels were measured with the real-time RT-PCR and normalised to β-actin using the $\Delta\Delta C_t$ method of relative quantification. Controls without RT and with no template cDNA were performed with each assay and all samples were run in triplicate. Data are reported as means ± S.E.M., $^{*}P < 0.05$, $^{**}P < 0.01$ compared to control.

administered to rats, DHEA (even in low doses) decreases epididymal adipose tissue mass and cellularity or reduces weight gain by inhibiting fat accumulation [4–6,8]. In lean rats, the fat-reducing effect of DHEA is not mediated by a reduction in caloric intake [4,8]. In our studies, after short-term treatment with DHEA, the rats had reduced epididymal adipose tissue mass (by 24%) and body weight (by 17%), which occurred despite no difference in food intake [23]. Adipose tissue is very sensitive to circulating DHEA and seems a potential target tissue for this adrenal steroid. Over recent years, white adipose tissue has been shown to secrete a variety of protein factors and hormones, notably adiponectin and leptin, involved in many aspects of organism physiology [10–13]. Maeda et al. demonstrated that adiponectin mRNA is the most abundant gene transcript in adipocytes [10], which makes adiponectin the main hormone secreted by adipose tissue. In humans, plasma adiponectin concentrations decrease with obesity [14]. Furthermore, an elevation in plasma adiponectin levels after weight reduction has been demonstrated [15]. Since changes in adipose tissue mass may influence the endocrine functions of this tissue, we focused on the effect of DHEA as a fat-reducing hormone on adiponectin gene expression and secretion from rat WAT. We observed that DHEA up-regulated adiponectin gene expression in adipose tissue more than two-fold (Fig. 1A). Serum adiponectin levels in DHEA-treated rats were also elevated indicating increased adiponectin secretion from adipose tissue (Fig. 2A).

The cellular mechanism of action of DHEA is still unclear. Recently, Ripp et al. have proposed that DHEA may act through a nuclear pregnane X receptor (PXR) [29]. However, in vitro studies revealed that DHEA and its metabolites are weak activators of this receptor. DHEA can be converted to a more potent androgen, testosterone; nevertheless, in our studies there were no significant differences in circulating testosterone levels after DHEA treatment (744 ng/ml in control versus 766 ng/ml in DHEA-treated rats) [23]. Thus, it seems unlikely that DHEA exerted its effects via activation of androgen receptors. Previous studies have revealed that DHEA acts as a peroxisome proliferator able to induce many genes through peroxisome proliferator-activated receptors (PPAR) [22]. In adipose tissue the predominant isoform of PPAR is PPARγ, which regulates the transcription of many adipocyte-specific genes [30]. PPARγ is a nuclear hormone receptor and a ligand-activated transcription factor that binds specifically to PPAR response elements in the promoter regions of target genes and activates their transcription [30]. PPARγ requires other nuclear proteins in order to function, i.e. RXRα. Recently, a functional PPAR-responsive element in the promoter region of the gene encoding adiponectin has been identified [21]. It has also been demonstrated that PPARγ/retinoid X receptor (RXR) heterodimer binds directly to the PPRE and increases adiponectin promoter activity in adipocytes [21]. Moreover, adiponectin expression and secretion are increased by activators of PPARγ [31]. These experimental observations suggest that PPARγ plays a significant role in the transcriptional activation of adiponectin gene expression via the PPRE in its promoter. Thus, in an attempt to clarify the molecular mechanism underlying the induction of adiponectin gene by DHEA, we aimed to determine the effect of this steroid hormone on PPARγ and RXRα gene expression in adipose tissue. DHEA induced PPARγ gene expression by over 2.5-fold and RXRα gene expression tended to be higher in adipose tissue of DHEA-treated rats (Fig. 1). A corresponding increase in PPARγ and adiponectin mRNA expression (Fig. 1D) suggests that PPARγ may be involved in the inductive effect of DHEA on adiponectin gene expression in adipose tissue; however, the exact mechanism of this effect needs further investigation.

In our studies DHEA administration had opposite effects on adiponectin and leptin gene expression and secretion from adipose tissue. In WAT of DHEA-treated rats, leptin gene expression tended to be lower [23]. Moreover leptin levels in serum were decreased by approximately 40% (Fig. 2B). Leptin gene expression and circulating leptin levels are regulated by adiposity, and they are reduced by the decrease in adipose tissue mass and weight reduction [13]. In our experiment the decrease in serum leptin levels is probably the consequence of the concomitant reduction in adipose tissue mass and leptin gene expression observed after DHEA treatment. A similar reduction in weight of adipose tissue accompanied by a decrease in plasma leptin levels has been observed previously in genetically obese rats fed a DHEA-containing diet [5,24]. Furthermore, leptin levels in serum, which reflect the size of fat tissue, were negatively correlated with circulating adiponectin levels (Fig. 2C). Leptin has been shown to be a negative target of PPARγ [32,33]. Heterozygous PPARγ-deficient mice showed over-expression and hypersecretion of leptin despite the smaller size of adipocytes and decreased fat mass [32]. Moreover, administration of rosiglitazone, a PPARγ ligand, reduced leptin mRNA levels in rats in a dose-dependent manner despite increased food intake and adipose tissue weight [33]. In contrast, PPARα activators, such as clofibrate and fenofibrate, had no significant effect on leptin mRNA levels in adipose tissue [25,33]. Therefore, an increase in PPARγ gene expression, observed in our studies in adipose tissue of DHEA-treated rats, may contribute, at least in part, to the down-regulation of leptin gene expression in rat WAT. Taken together, the presented observations suggest that PPARγ may act as a common *trans*-regulatory factor involved in adiponectin and leptin gene expression in adipose tissue and may regulate the transcription of both genes in a reverse manner.

PPARγ is known to increase adipogenesis, it could be argued that up-regulation of this transcription factor is therefore likely to result in an increase in adipose tissue mass. Nevertheless, DHEA treatment leads to a decrease in adipose tissue mass and adipocyte cellularity [4–8,23].

One possible explanation for this could be the induction of resistin gene by DHEA [23]. Resistin, an adipocyte-derived hormone, is suggested to function as an inhibitor of adipocyte differentiation [34]. The enhanced expression of resistin may influence the proliferation and differentiation of adipocytes and contribute to the reduction in adipose tissue mass after DHEA treatment. This is in agreement with the results published by Lea-Currie et al., who found that DHEA reduces preadipocyte proliferation and differentiation [35].

An increase in PPARγ gene expression after DHEA administration is not in line with the study by Kajita et al., showing a decrease in PPARγ protein amount in adipose tissue of genetically obese rats after 2 weeks of DHEA treatment [5]. However, the level of PPARγ was abnormally elevated in adipose tissue of obese rats used in their study and the authors did not investigate the effect of DHEA on PPARγ protein levels and/or mRNA levels in adipose tissue of lean rats. Gorla-Bajszczak et al. have shown previously that PPARγ mRNA levels in adipose tissue of obese and diabetic rats are more than two times higher than in lean rats [36]. Since elevated levels of PPARγ could mask a potential effect of DHEA on adiponectin and leptin gene expression, in our studies lean rats were used.

Recently, Yamauchi et al. cloned two different isoforms of adiponectin receptor (AdipoR1 and AdipoR2) [37]. Both isoforms are expressed in many cell types [37,38]. Furthermore, it has been shown that in human macrophages adiponectin receptor gene expression is regulated by PPAR [39]. Thus, we aimed to determine the effect of DHEA administration on AdipoR1 and AdipoR2 gene expression in rat adipose tissue. We found that DHEA induced an approximately three-fold increase in the expression of AdipoR1 (Fig. 3A); however, it had no significant effect on AdipoR2 (Fig. 3B). An accompanying increase in the expression of PPARγ suggests that in adipose tissue the AdipoR1 gene may be regulated by this transcription factor (Fig. 3C). To our knowledge this is the first report of adiponectin receptor gene expression and the putative role of PPARγ in its regulation in adipose tissue.

Since adiponectin receptors are expressed in adipocytes, adiponectin may play an important role in the regulation of adipose tissue metabolism via autocrine and/or paracrine manner. In vitro studies revealed that both isoforms of adiponectin receptor can mediate increased AMPK phosphorylation and PPARα ligand activity by adiponectin binding, thus activating mitochondrial fatty acid oxidation and glucose uptake [37]. In our studies the rate-limiting enzyme of mitochondrial fatty acid oxidation pathway, LCPT-1, was up-regulated in adipose tissue of DHEA-treated rats (Fig. 4A). Moreover, the expression of HSL was induced in response to DHEA administration (Fig. 4B). HSL is a key lipolytic enzyme in adipose tissue, thus its up-regulation may be one of the reasons for fat reduction seen after DHEA treatment. In contrast, FAS (which plays a central role in de novo lipogenesis) gene expression tends to be lower in WAT of rats fed a DHEA-containing diet (Fig. 4C).

It has been found previously that DHEA stimulates resting metabolic rate and lipid oxidation [8]. After 2-week administration of DHEA we observed an increase in brown adipose tissue (BAT) mass by 37% [23]. Moreover, UCP-1 gene expression in brown adipose tissue of DHEA-treated rats tended to be higher, suggesting an increased thermogenesis rate (not shown). This is in line with previously published studies [6,7]. Elevations in BAT mass and activity are associated with an increase in fatty acid oxidation and energy expenditure [40]. Thus, proposed mechanisms for the decrease in fat mass and lower weight gain resulting from DHEA treatment may involve an increase in lipolysis rate, increased flux of fatty acids through the β-oxidation pathway and a decrease in de novo lipogenesis rate in adipose tissue, accompanied by an increase in energy expenditure.

Previous studies have shown that DHEA supplementation has beneficial effects in animal models of diabetes [4,41] and atherosclerosis [42,43]. DHEA inhibited atherogenesis in injured and transplanted vessels [42,43], therefore, an anti-atherogenic effect of DHEA was presumed. Furthermore, DHEA had a protective effect against development of insulin resistance in rats fed a high fat diet and restored insulin sensitivity in genetically obese Zucker fatty rats [4,41]. Last year Villareal and Holloszy reported a significant increase in an insulin sensitivity index in response to DHEA in elderly persons [9]. We demonstrated here that DHEA administration to the rats increases the circulating levels of adiponectin. It has been shown that this adipocyte-specific secretory protein has anti-atherogenic properties. Adiponectin acts as a negative regulator of the endothelial adhesion molecule expression, reduces lipid accumulation in monocyte-derived macrophages and macrophage-to-foam cell transformation as well as strongly suppresses proliferation and migration of vascular smooth muscle cells [18–20]. Furthermore, in various animal models, adiponectin increases insulin sensitivity and improves glucose tolerance [16,17]. In humans, plasma adiponectin concentrations decrease with obesity and are positively correlated with whole-body insulin sensitivity. Recently, an association between adiponectin concentrations in plasma and risk of type 2 diabetes in apparently healthy individuals has been reported [44]. Moreover, there is increasing evidence that genetic variants in the adiponectin gene itself and/or in genes encoding adiponectin regulatory proteins, such as PPARγ, are associated with hypoadiponectinaemia, insulin resistance and type 2 diabetes, indicating that this naturally circulating adipocytokine may play a preventive role against the development of type 2 diabetes. Thus, in view of our results, the beneficial effect of DHEA on vascular wall and insulin sensitivity in vivo might be exerted indirectly, through an activation of adiponectin gene expression and secretion by adipose tissue.

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