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Comparative assessment of estrogenic responses with relevance to the metabolic syndrome and to menopausal symptoms in wild-type and aromatase-knockout mice

Manuela I. Bader*, Jannette Wober, Georg Kretzschmar, Oliver Zierau, Günter Vollmer

Institute of Zoology, Chair of Molecular Cell Physiology and Endocrinology, Technische Universität Dresden, 01062 Dresden, Germany

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ABSTRACT

Knockout of the Cyp-19 gene (aromatase) renders mice to have insufficient endogenous estrogen production and contributes to the development of symptoms related the metabolic syndrome, including excess adiposity and insulin resistance. This study comparatively assessed the estrogen responsiveness in animal models of genetical versus surgical (ovariectomy) origin of estrogen deficiency. Evaluation of physiological parameters and gene expression pattern in response to estrogens revealed differences in estrogen responsiveness between aromatase deficient and castrated or intact wild-type mice. ArKO mice had a significantly higher bodyweight than matched ovariectomized wild-type mice. The weight of the completely regressed uterus following ovariectomy was higher than the uterine weight of ArKO mice. Further, alterations in metabolic parameters like increased serum leptin levels and decreased plasma glucose levels in genetically deficient mice became apparent. Finally, expression pattern of estrogen responsive genes differed in the two experimental models of estrogen deficiency. Both, in uterine and adipose tissues the regulation of expression of some genes either was inversed of regulation or considerably differed in the magnitude of the response in the two models. Our studies demonstrate that the cause of estrogen deficiency significantly impacts on estrogen responsiveness and may be of relevance for investigations on aspects of estrogen deficiency and metabolic and/or menopausal symptoms.

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1. Introduction

Systemic cessation of estrogen production during the climacteric transition, amongst numerous other symptoms, is associated with increased adiposity and greater risk to develop a metabolic syndrome (MetS) [1]. To reduce symptoms hormone replacement therapy with estrogens and/or progestins or estrogen-like plant derived substances are commonly used. To test the efficacy and safety of compounds, the ovariectomized (ovx) rodent model is regarded as the appropriate preclinical experimental model to investigate the role of estrogens on mechanisms and consequences of substitution of hormone deficiency.

Aromatase is encoded by the *Cyp19* gene and catalyzes the final step in the biosynthesis of C18 estrogens from C19 steroids. Independently, Fisher et al. [2] and Toda et al. [3] developed a mouse model, the so-called aromatase-knockout (ArKO)-mouse, representing estrogen insufficiency by disrupting the aromatase gene.

The human aromatase encoding gene had been cloned, characterized and found to be atypical compared to genes encoding other P450 enzymes. There are a number of untranslated regions in the first exon which occur in aromatase transcripts in a tissue-specific fashion, due to differential splicing as a consequence of the use of tissue-specific promoters [4]. As a result of the aromatase knockout, testosterone levels are markedly elevated in the female ArKO mice, to values approximately 10 times above those of wild-type (WT) females [2]. This is one of the factors contributing to infertility of female ArKO mice. A hallmark of estrogen ablation by castration is the regression of the uterus to approximately 20–25% of its original weight and reconstitution of the uterine weight in response to estrogenic compounds [3]. In general ArKO mice have a heavier bodyweight and they have more adipose tissue (AT) than their WT littermates. The uterine size of ArKO mice compared with uterine size of WT is diminished [3].

For all those reasons, ArKO mice seem to be a useful experimental model to study the effectiveness and safety of compounds, intended to be used in either estrogen replacement therapy, comprising estrogens themselves or natural compounds exhibiting estrogen-like properties.

Abbreviations: vt, vehicle treated (DMSO and castor oil); ovx, ovariectomized; ArKO, aromatase-knockout; AT, adipose tissue; WT, wild-type; MetS, metabolic syndrome; PCR, polymerase chain reaction; s.c., subcutaneous; ELISA, enzyme-linked immunosorbent assay; Esr1, estrogen receptor alpha; Esr2, estrogen receptor beta; E_2, estradiol.

* Corresponding author. Tel.: +49 351 463 34733; fax: +49 351 463 31923.
E-mail address: manuela.bader@tu-dresden.de (M.I. Bader).

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Some investigators associate the ArKO mouse with other problems resulting from estrogen deficiency during menopausal transition, such as increasing amount of AT and an ovarian phenotype. The ovarian phenotype is characterized by blocked folliculogenesis, hemorrhagic cysts and development of cells which phenotypically resemble Sertoli cells [5–8]. However, it is worth mentioning that ArKO mice are unable to produce estrogen throughout their entire life span, which may render them different to ovx WT-mice. While ovx WT-mice have been through the estrogen dependent developmental program of reproductive organs in embryogenesis and only later set into a state of estrogen depletion following their castration.

Hence, our aim was to assess comparatively the effects of estrogen treatment in genetically estrogen-deficient ArKO mice and their intact WT as well as their ovx WT counterparts respectively, with the latter being used as control animals following surgical estrogen depletion. Besides tissue weights, we focused on the organ specific assessment of mRNA expression pattern of estrogen responsive genes in both the uterus and AT. In order, to obtain more detailed information on estrogen action we performed gene expression analyses to assess comparatively the general estrogen responsiveness, estrogenic regulation of genes related to pathophysiological alterations in MetS and expression levels of genes potentially representing a mechanistic link between estrogen deficiency and MetS.

2. Materials and methods

2.1. Mice

The ArKO mice were kindly provided by Prof. Dr. Evan Simpson (Melbourne, Australia). Originally they were generated by disrupting the *Cyp19* gene as described by Fisher et al. [2]. The mice had been crossed back to a C57BL/6J background for 10 generations. Heterozygous females and males were breed and their homozygous aromatase-null-offspring as well as their WT siblings were used for the following experiments. Thereby the studied animals identical in their genetic and epigenetic background with the exception of the knockout of the *Cyp19* gene and the castration of the WT animals. Female mice were genotyped by PCR [9]. Animals were maintained under controlled conditions of temperature ($20 \pm 1\,^{\circ}$C, relative humidity 50–60%), illumination (12 h light, 12 h dark), water *ad libitum* and phytoestrogen free diet (Harlan 2019 Rodent Breeding, Harlan Winkelmann, Borchen, Germany). All animal handling and all experimental conditions were licensed and carried out according to the Institutional Animal Care and Use Committee guidelines as regulated by the German federal law governing animal welfare.

2.2. Experimental procedure

Adult female ArKO mice and matched female homozygote WT littermates were then randomly assigned to four experimental groups consisting of seven animals per group, as illustrated in Table 1. One group of the WT-mice was bilaterally ovariectomized under general anesthetic conditions on day 80 for estrogen ablation. The other group of intact WT-mice served as estrogenized positive control. This unusual experimental setup was selected to adhere closely to the 3R principle of animal welfare and to avoid enclosure of unnecessary additional experimental groups. After 14 days of endogenous hormonal decline in the ovx WT group, these animals as well as intact WT and one of the ArKO group were treated with the vehicle (i.e. solvent and castor oil) subcutaneously (s.c). The treated group of the ArKO mice was substituted s.c. with 17β-estradiol (E_2) (10 μg/kg/BW/d) (Sigma–Aldrich, Munich, Germany) for three days. As mentioned above cycling WT animals served as second group of estrogenized animals.

2.3. Tissue collection

Mice were sacrificed by cervical dislocation, followed by blood collection and necropsy. The blood was allowed to clot. In addition, serum and plasma were collected and stored at $-20\,^{\circ}$C. At necropsy, uterus and gonadal AT were removed and immediately stored in liquid nitrogen for future use.

2.4. Measurement of plasma glucose and serum leptin levels

The glucose oxidase method was used to determine glucose concentrations in the plasma using a quantitative colorimetric glucose determination Kit (QuantiChrom™ Glucose Assay Kit, BioAssay Systems, Hayward, USA) at 630 nm. Furthermore, leptin concentrations were determined in serum by an *in vitro* enzyme linked immunosorbent assay (ELISA) for the quantitative measurement of mouse leptin (RayBio® Mouse Leptin ELISA Kit (RayBiotec Inc., Norcross, USA).

2.5. RNA sample preparation and real-time PCR

Total RNA was isolated from 30 mg tissue of uteri and gonadal AT using Qiagen RNeasy®Plus (Qiagen GmbH, Hilden, Germany). RNA from the same treatment group was pooled and the DNA contaminations were enzymatically eliminated by digestion (Desoxyribonuclease 1, Roche Diagnostic GmbH, Mannheim, Germany). Oligo$(dT)_{18}$-primers and MMLV-Reverse Transcriptase (Roche Diagnostic GmbH, Mannheim, Germany) were used for the first-strand cDNA synthesis. Following amplification by real-time PCR, relative mRNA levels were detected by SYBR green fluorescence method using the iCycler Thermal Cycler with iQ real-time Detection System (BIO-Rad Laboratories GmbH, Munich, Germany) as previously described [10]. Ribosomal protein S 18 (*Rps18*) rRNA expression was used as an internal reference gene. Fold expression was calculated using the $2^{-\Delta\Delta Ct}$ method [11]. Table 2 shows primer pair sequences and their respective amplicon size

2.6. Statistical analysis

Gene expression data are presented as mean ± SD for all PCRs. All PCRs were conducted in triplicates with three independent cDNA samples. Statistical analysis was performed for weight measurements by ANOVA (one-way) followed by Bonferronie post hoc test, PCR data were analyzed using Student's *t*-test.

3. Results

3.1. Body and uterus wet weights

Body weights were determined prior and after the treatment. As shown in Table 3, both the vehicle-treated (vt) and the E_2-treated ArKO mice had significant heavier body masses than the both WT mice groups. The uterine masses of the vt ArKO mice were significantly lower than those measured in vt ovx WT mice. However, the surgical procedures were effective as the uteri of the vt ovx WT-mice regressed and resulted in a significantly and about 5-fold lower uterine wet weight if compared to those of the vt intact WT-mice. In addition, ArKO mice responded well to E_2-treatment by an approximately 10-fold increase in the uterine wet weight compared to vt ArKO mice.

Table 1
Experimental design.

	Treated mice ($n = 7$)	Untreated mice ($n = 7$)	Untreated mice ($n = 7$)	Treated mice ($n = 7$)
Estrogen status	Surgical estrogen deficiency (ovariectomized)	Endogenous E_2 production	Genetical estrogen deficient	Estrogenized followed by injection of E_2
Animals	WT-mice	WT-mice	ArKO mice	ArKO mice
Treatment	Vehicle	Vehicle	Vehicle	17β-Estradiol
Chow/duration	Phytoestrogen free/3 days s.c.			

Table 2
Primer pair sequences and respective amplicon size in base pairs (bp).

Transcript	Sequence		Product size(bp)
	Forward primer	Reverse primer	
Estrogen response markers			
Esr1	TGT TTG CTC CTA ACT TGC TCC C	GGT GGA TGT GGT CCT TCT CTT	222
Esr2	GCC AGG AAG CAG AGA GTA GC	TCA TGC TGA GCA GAT GTT CC	189
Pgr	CTG GCA TGG TCC TTG GAG	TGG AAG TGT CAG GCT TTG TG	246
Ltf	GCA AAA CCA CAT CGG AGA AG	GGG AGT GAG GAG ACC AGA TG	204
Genes related to pathophysiological alterations in MetS			
Lep	AGA TCC CAG GGA GGA AAA TG	TGA AGC CCA GGA ATG AAG TC	217
Lepr	TCG ACA AGC AGC AGA ATG AC	CTT GTG CCC AGG AAC AAT TC	159
Insr	GAA TGT GGG GAT GTC TGT CC	ATC AGG TTC CGA ACA GTT GC	201
Genes that might link between estrogen deficiency and MetS			
Pparg	TCA TGA CCA GGG AGT TCC TC	CAG GTT GTC TTG GAT GTC CTC	200
Tnfa	GGC CTC CCT CTC ATC AGT TC	GGT TGT CTT TGA GAT CCA TGC	178
Il6	TGA ACA ACG ATG ATG CAC TTG	CTC TGA AGG ACT CTG GCT TTG	264
Internal reference gene			
Rps18	AGG ATG TGA AGG ATG GGA AG	TTG GAT ACA CCC ACA GTT CG	187

Esr1, estrogen receptor alpha; *Esr2*, estrogen receptor beta; *Pgr*, progesterone receptor; *Ltf*, Lactoferrin; *Lep*, leptin; *Lepr*, leptin receptor; *Insr*, insulin receptor; *Pparg*, peroxisome proliferator-activated receptor gammma; *Tnfa*, tumor necrosis factor alpha; *Il6*, interleukin-6; *Rps18*, ribosomal protein S 18.

Table 3
Bodyweight and uterus wet weights of ovx and intact WT mice as well as untreated and E_2-treated ArKO mice.

Group	Body weight (g)	Uteruswet weight (g/kg BW)
Vehicle-treated ovx WT mice	20.9 ± 1.3	1.07 ± 0.2
Vehicle-treated intact WT mice	19.9 ± 1.7	$5.18 \pm 3.0^{**}$
Vehicle-treated ArKO mice	$22.7 \pm 3.1^{**}$	$0.28 \pm 0.1^{*}$
E_2-treated ArKO mice	$24.3 \pm 1.7^{+++}$	$2.90 \pm 1.1^{\#\#\#}$

Results are presented as mean ± standard deviation ($n = 7$).
$^{*}p < 0.05$, $^{**}p < 0.01$ as compared to vt ovx WT-mice.
$^{+++}$ $p < 0.001$ as compared to vt intact WT-mice.
$^{\#\#\#}$ $p < 0.001$ as compared to vt ArKO mice.

3.2. Plasma glucose and serum leptin levels

Serum leptin levels were significantly elevated in the ArKO mice compared to the WT mice (Table 4).

Comparing serum leptin levels between vt ovx WT mice with vt intact WT mice and between vt ArKO mice with E_2-treated ArKO mice estradiol treatment seems to elevate the serum leptin production.

Table 4
Serum–leptin and plasma–glucose-levels of ovx and intact WT mice as well as untreated and E_2-treated ArKO mice.

Group	Serum–leptin-level	Plasma–glucose-level
Vehicle-treated ovx WT mice	59.7 ± 24.4	124.4 ± 51.3
Vehicle-treated intact WT mice	93.3 ± 77.8	114.3 ± 36.5
Vehicle-treated ArKO mice	$127.6 \pm 55.1^{*}$	96.1 ± 41.6
E_2-treated ArKO mice	$145.1 \pm 76.6^{+}$	96.2 ± 34.4

Results are presented as mean ± standard deviation ($n = 7$).
$^{*}p < 0.05$, $^{**}p < 0.01$ as compared to vt ovx WT-mice.
$^{+}$ $p < 0.05$ as compared to vt intact WT-mice.

No statistically significant differences in plasma glucose levels were measured within the four groups (Table 4). However, plasma glucose levels appear to be lower in ArKO than in WT mice.

3.3. Gene expression analysis

3.3.1. Uterus

The aim was to compare E_2 action/function in vt ovx and vt intact WT mice to vt and E_2-treated ArKO mice. The key question addressed was to elucidate whether there is a functional difference in estrogen action following surgical estrogen ablation (modeling the menopause situation) and genetical estrogen depletion. As readout served molecular parameters related to reproductive organ function and those related to aspects of the MetS. To obtain hints towards potential modification of reproductive organ function the uterine gene expression levels of selected genes (Table 2) were assessed and compared.

Considerable alterations of gene expression levels and depending on the experimental approach used were found for the mediators of the estrogenic response estrogen receptor alpha (*Esr1*), estrogen receptor beta (*Esr2*) and their response genes lactoferrin (*Ltf*) and progesterone receptor (*Pgr*) as well as for the peroxisome proliferator-activated receptor gamma (*Pparg*), a molecule potentially linking estrogen deficiency and MetS (Fig. 1). To assess E_2 function in WT and ArKO mice the situation in vt intact WT and in E_2-treated ArKO mice was compared to vt ovx WT and vt ArKO mice (mRNA expression level set to 1), respectively (Fig. 1B). The mRNA expression levels of the vt ovx WT and vt ArKO mice are shown as $2^{-\Delta ct}$ values (Fig. 1A) normalized to the housekeeping gene *Rps18*. Although differences in the basal expression levels of genes of interest are seem to exist, only those for Esr1 reached the level of statistical significance. The gene expression levels of *Esr1*, *Pgr*, and *Pparg* mRNA were significantly decreased in response to the presence of E_2 if compared to the respective reference groups.

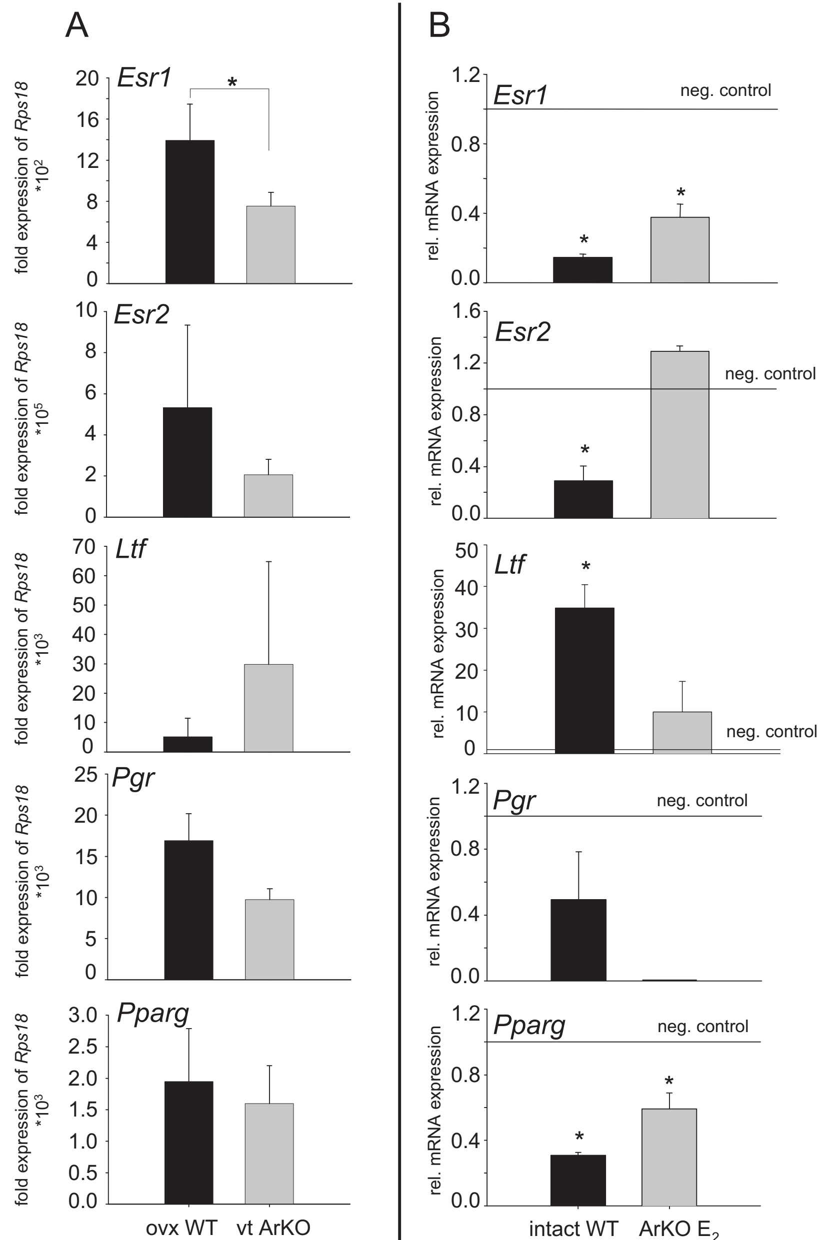

Fig. 1. Uterine gene expression of *Esr1*, *Esr2*, *Pgr*, *Ltf*, and *Pparg* in WT and ArKO mice. In (A) the mRNA expression levels of the vt ovx WT and vt ArKO mice are shown as $2^{-\Delta ct}$ values normalized to the housekeeping gene *Rps18*. These values represent basic expression levels in non-estrogenized conditions in the two experimental models compared. In (B) relative mRNA expression levels of vt intact WT and ArKO E_2 mice are shown which were normalized to the corresponding non-estrogenized status of the animals (black horizontal line) namely vt ovx WT and vt ArKO mice, respectively. All gene expression data are presented as mean ± SD. Statistical analyses were performed using Student's *t*-test. $^*p < 0.05$.

Ltf expression was clearly increased by E_2 in WT and in mutant mice. All four regulated genes differed in the magnitude of the observed response comparing effectiveness of E_2 function in WT and ArKO mice. Regulation of *Esr2* expression showed a different response pattern for WT and ArKO mice. A significant down-regulation was observed in vt intact WT, but not in ArKO E_2 mice (Fig. 1B).

3.3.2. Adipose tissue

To study the context between estrogen effects and MetS gene expression levels of response genes in gonadal AT were also assessed and compared. Considerable differences in gene expression levels in vt WT mice and after E_2-treatment in ArKO mice (Fig. 2B) were found for the both estrogen receptor subtypes *Esr1* and *Esr2*. Similarly genes related to pathophysiological alterations in MetS leptin (*Lep*), leptin receptor (*Lepr*) and insulin receptor (*Insr*), as well as the genes potentially linking estrogen deficiency and MetS interleukin-6 (*Il6*), tumor-necrosis-factor alpha (*Tnfa*) and *Pparg* (Fig. 2B) showed differences in their expression levels.

As shown in Fig. 2A, basal expression levels of investigated genes do not differ significantly as indicated by $2^{-\Delta ct}$ values of vt ovx WT mice and vt ArKO mice with the exception of expression levels of IL-6. The gene expression levels (Fig. 2B) in response to E_2 in comparison to its estrogen deficient counterparts resulted in varying patterns. In ArKO mice the *Esr1* was up- and *Esr2* was down-regulated. In WT mice no regulation was observed.

Changes of expression levels of *Lep*, *Lepr* and *Insr* mRNA were similarly in both animal models, only the up-regulation of *Lepr*

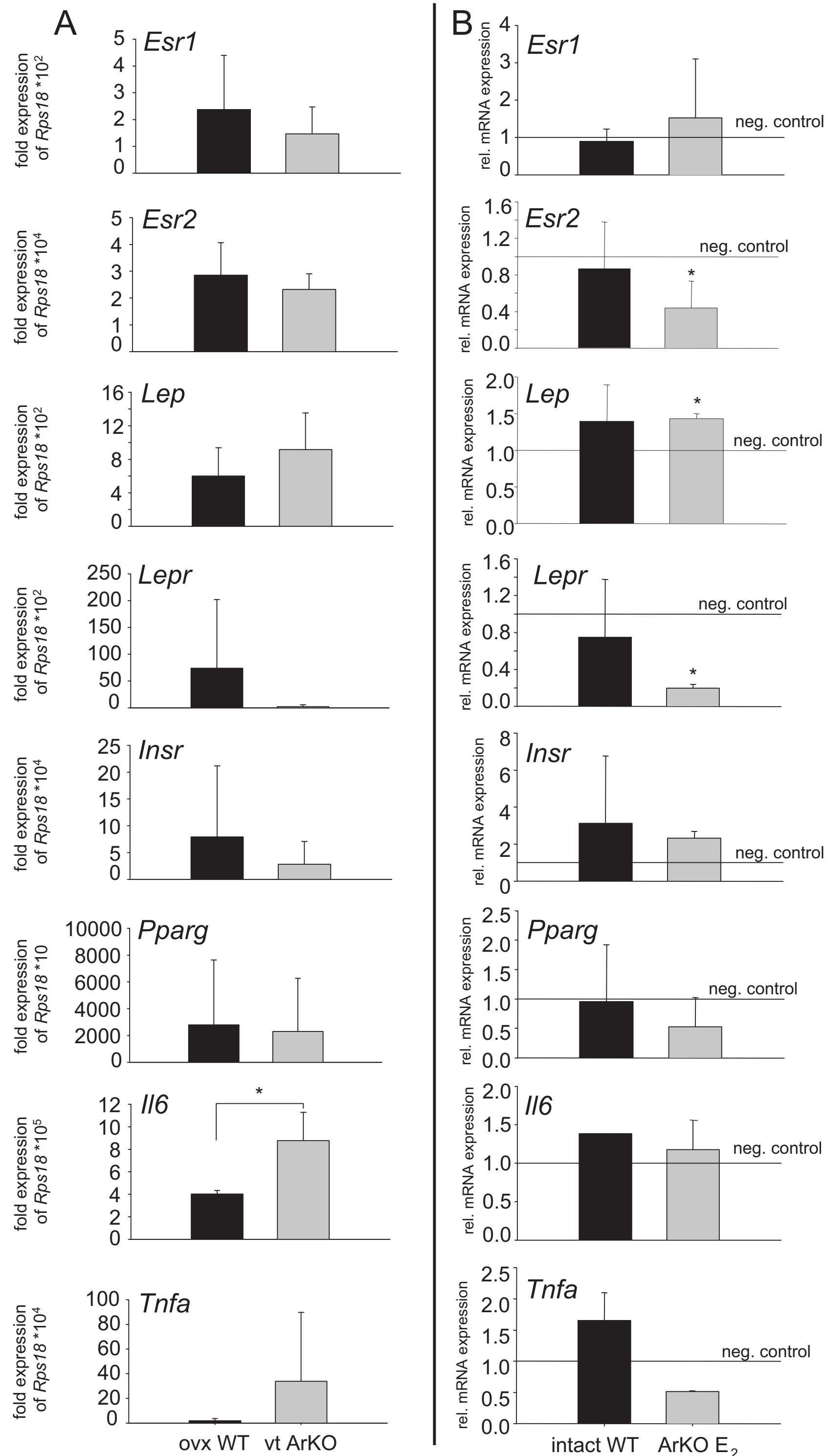

Fig. 2. Gonadal AT gene expression of *Esr1*, *Esr2*, *Lep*, *Lepr*, *Insr*, *Pparg*, *Il6* and *Tnfa* in WT and ArKO mice. In (A) the mRNA expression levels of the vt ovx WT and vt ArKO mice are shown as $2^{-\Delta ct}$ values normalized to the housekeeping gene *Rps18*. These values represent basic expression levels in non-estrogenized conditions in the two experimental models compared. In (B) relative mRNA expression levels of vt intact WT and ArKO E_2 mice are shown which were normalized to the corresponding non-estrogenized status of the animals (black horizontal line) namely vt ovx WT and vt ArKO mice, respectively. All gene expression data are presented as mean ± SD. Statistical analyses were performed using Student's *t*-test. $^*p < 0.05$.

mRNA was more pronounced in ArKO mice. *Pparg* is not regulated in vt WT but down-regulated in E_2 treated ArKO mice. However, the *Tnfa* mRNA levels were affected in opposite directions. A weaker up-regulation of the *Il6* mRNA level was observed in E_2-treated ArKO compared to vt WT-mice.

4. Discussion

The aim of the present study was to assess comparatively the effects of estrogens in two models of estrogen deficiency in an organ specific manner, analysing uterine tissue and AT. As genetic

model of estrogen deficiency the ArKO mouse model was used and estrogen action in this model was compared to estrogen function in the castrated mouse model. As physiological readouts body and organ weights were used and combined with molecular endpoints of estrogen action which were assessed by semi quantitative real-time PCR. For the latter we investigated the expression pattern of three classes of genes: known estrogen response markers, genes potentially involved in the development of features of the MetS and those genes that might connect estrogen deficiency with MetS.

4.1. Physiology

Adult female ArKO mice had a significant heavier body weight than WT mice which was expected and is in agreement with findings by Jones et al. [5]. Even the ovx WT mice slightly gained body weight in the comparably short period of 14 days of hormonal decline if compared to their intact WT littermates. This weight gain effect was also described for ovx rats [12] and in ERα-knockout-mice [13]. As described elsewhere [3] the uterus was underdeveloped in ArKO mice with only a weight of only 26–56% of the weight of uteri from ovx WT mice in our study. The effect of ovx apparently does not become a lot more pronounced if periods of hormonal decline exceeded 14 days [14,15]. In other words, uterine regression following castration does not result in uterine wet weights as low as observable in uteri of ArKO mice.

4.2. Metabolic considerations

It had been proposed that a decrease in serum leptin levels found at menopause was due to the decrease of estrogen levels [16]. Jones et al. demonstrated that the circulating serum leptin levels were significantly elevated in the ArKO mice, an observation shared by our findings. This observation was not surprising, given the established association between an obese stature and an increased production of leptin in most obese human subjects and most animal models of obesity. Leptin regulates body fat predominantly by decreasing food intake [5,6]. In our experiment, we found increased serum leptin levels in ArKO mice if compared to WT-mice.

Another result of our study showed that there were no differences of plasma glucose levels between the two models of estrogen deficiency, but a trend of a lower plasma glucose level in ArKO mice was apparent [5]. Takeda et al. described a similar observation in experiments with male ArKO mice and pointed out, that male ArKO mice had no significant difference in plasma glucose level compared to their WT littermates [17]. Interestingly and in trend, the plasma glucose levels in intact WT mice in comparison to ovx WT mice were reduced but none of the differences reached statistical significance.

4.3. Genexpression data

We present for the first time a comparative assessment of gene expression in two models of estrogen deficiency. In theory, if ArKO mice and ovx WT counterparts respond to estrogens in a similar way, this should result in a similar pattern of regulation of estrogen responsive genes. Figs. 1 and 2 compare the estrogen responsiveness observed in the two deficiency models. It becomes obvious that the regulation of expression of most of the investigated genes was not identical in the two model of deficiency (Figs. 1 and 2). Speaking generally, the major differences of the results of the comparative assessment of estrogen regulated gene expression obtained from the two estrogen deficiency models can be clustered in two groups. One observed feature was that changes of expression occurred in the same direction but differed considerably in the magnitude. Differences along this lane may in part be due to differing levels of expression of the investigated genes in the two different experimental approaches (panel A of Figs. 1 and 2). The second group to which estrogenic response pattern can be clustered represents genes which responded to 2 treatment in an inverted pattern of expression.

In the uterus, estrogen response markers like *Esr1* and *Ltf* mRNA expression were found to be significantly up-regulated in the presence of estradiol in both experimental approaches, but the magnitude of the effect clearly differed. Conversely, *Esr2* expression in ArKO mice was increased by E_2 thereby responded to the presence of estradiol by an up-regulation whereas in intact WT mice showed lower expression levels than castrated counterparts, strongly resembling the situation in the ovx rat model [18]. The results observed for *Ltf* correspond with those previously published by Toda et al. [3]. Our results on *Pgr* expression appear contradictory to findings in the literature [3] as we found a down regulation of *Pgr* mRNA in ArKO mice by the presence of estradiol in our three-day uterotrophic assay. Similar findings we published in the ovx rat model which showed by a time dependency of *Pgr* mRNA expression in the uterotrophic assay in response to E_2 treatment, following a defined temporal pattern. *Pgr* expression increased after 7 h of stimulation, decreases later on and at 72 h following treatment to levels even below those in ovx controls [19–21]. Theoretical causes, which may explain this finding, are the age of the mice, which were with 35 days much younger in the study of Toda et al., than those used in our study. Further, there are major differences in dosage and treatment periods. Toda et al. used 15 μg of estradiol per animal every four days, which means around 1100 μg/kg per injection, which is exceedingly high (about 110 times higher then our E_2 levels). In addition, we adapted our approach to OECD recommendations and performed a three-day assay whereas the other study had an observation period of 28 days [3].

Adipose tissue in general has long been considered a mere storage site for lipids and therefore an energy reservoir for the maintenance of vital cell functions [22,23]. Systemic loss of estrogen at menopause was associated with increased adiposity, which was implicated in the elevated risk of age-related metabolic diseases in women [24]. Estrogen has direct effects on AT, for example ovariectomy decreased lipolysis in AT. D'Eon et al. provided evidence that E_2 has significant effects on energy metabolism through both genomic and non-genomic mechanisms of ER-pathways and that these effects collectively promote leanness in ovx WT mice [5,24]. This observation proves that the observed consequences following disruption of the aromatase gene *Cyp19* on a molecular level [3]. *Tnfa* is an indicator for insulin resistance and *Pparg* known to be a modulator of leptin production, as well as an insulin sensitizer. The expression of these genes was evaluated because changes of their expression pattern may mechanistically link estrogen deficiency to MetS. *Tnfa* showed a higher mRNA expression level in gonadal AT of WT-mice if compared to gonadal AT of ovx counterparts however, it was down-regulated in the ArKO model after E_2 treatment. *Tnfa* was found to be associated with insulin resistance and Diabetes Type 2 in obesity. A positive correlation was observed between levels of its mRNA expression in subcutaneous AT and insulinemia in women. Also, obese patients with insulin resistance (especially women) had an increased *Tnfa* secretion [25]. The down-regulation seen in response to E_2 treatment in ArKO mice may be caused either by the species variations or by the fact that estradiol was missing during organ development of the animals which may impact on responsiveness to estradiol later in life. Otherwise our findings on *Tnfa* and *Il6* are in agreement with findings in the literature by Riant et al. [26].

In conclusion, using physiological parameters of organs or whole organisms, evaluation of gene expression pattern and immunobiochemical assessment of serum parameter we were able to show that estrogen deficient ArKO mice respond differentially to estro-

gens if compared to the estrogenic response pattern detectable in their intact and ovx WT counterparts. Based on these observations and as a working hypothesis for future investigations, we suggest to use ovx rodents as an experimental model for any studies which focus on the investigation of substances intended to be used to treat general menopausal symptoms. On the other hand, the ArKO mouse seems to be highly suitable model to investigate questions which link estrogen deficiency to symptoms of the MetS, a question which is relevant for approximately 40% of all menopausal women [1].

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