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Soy supplementation: Impact on gene expression in different tissues of ovariectomized rats and evaluation of the rat model to predict (post) menopausal health effect

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ABSTRACT

This toxicogenomic study was conducted to predict (post)menopausal human health effects of commercial soy supplementation using ovariectomized rats as a model. Different target tissues (i.e. breast, uterus and sternum) and non-target tissues (i.e. peripheral blood mononuclear cells (PBMC), adipose and liver) of ovariectomized F344 rats exposed to a commercially available soy supplement for eight weeks, were investigated. Changes in gene expression in these tissues were analysed using whole-genome microarray analysis. No correlation in changes in gene expression were observed among different tissues, indicating tissue specific effects of soy isoflavone supplementation. Out of 87 well-established estrogen responsive genes (ERGs), only 19 were found to be significantly regulated ($p < 0.05$) in different tissues, particularly in liver, adipose and uterus tissues. Surprisingly, no ERGs were significantly regulated in estrogen sensitive breast and sternum tissues. The changes in gene expression in PBMC and adipose tissue in rats were compared with those in (post)menopausal female volunteers who received the same supplement in a similar oral dose and exposure duration in human intervention studies. No correlation in changes in gene expression between rats and humans was observed. Although receiving a similar dose, in humans the plasma levels expressed as total free aglycones were several folds higher than in the rat. Therefore, the overall results in young ovariectomized female F344 rats indicated that using rat transcriptomic data does not provide a suitable model for human risk or benefit analysis of soy isoflavone supplementation.

1. Introduction

The advances in health care and the public initiative towards healthy living have increased the number of women in the (post)menopausal age group worldwide. The menopause transition results in declining ovarian functions and leads to significant hormonal changes in the female body, in particular in the reduced production of the female hormone estrogen. As a result women in this age group face several physical and mental difficulties including hot flashes, night sweats, vaginal atrophy, and an increased risk of developing osteoporosis. Menopause is inevitable and many women during their menopausal period take hormone replacement therapy (HRT) or use soy based dietary supplements. These soy based supplements contain phenolic plant compounds called isoflavones [1,2] which have structural similarities with estradiol [3]. Despite inconsistencies among the available data, there is growing evidence supporting the notion that soy isoflavones are effective in the reduction of menopausal symptoms [4,5]. Therefore, the popularity and availability of soy supplements are increasing, although concern exists within governmental and public health related organizations [6,7] regarding the safety of long-term exposure to self-administrated high levels of soy isoflavones, which may exceed the levels that can be obtained from the diet.

The effects of soy isoflavones are thought to be induced via estrogen receptors (ERs) of which an alpha (ERα) and beta (ERβ) form exist. soy

Abbreviations: ERGs, estrogen responsive genes; ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; PBMC, peripheral blood mononuclear cells

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isoflavones may regulate transcription of ER target genes via the estrogen-responsive elements (EREs) present within gene promoter regions in target tissues. It has been shown that ERα activation stimulates cell proliferation, whereas ERβ activation leads to inhibition of proliferation and stimulation of apoptosis [8–10]. It is known that the relative occurrence of ERα and ERβ is tissue dependent. For example, ERα is predominant in the mammary gland, the epididymis, testis, uterus, kidney and the pituitary gland, whereas ERβ is more present in the prostate, bladder and lungs [11–14]. When (post)menopausal women produce small amounts of endogenous estradiol, it is possible that binding of SIF from food supplements to ERs may produce estrogen-responsive effects especially in ERβ-sensitive target tissues, because soy isoflavone have been shown to be particularly ERβ-active [10,11]

During the past decades, the application of microarray technology has opened up new opportunities to study the effects of food and food supplements in the control of cellular processes and related health effects upon exposure to different type of compounds [15–17]. By using transcriptomic techniques it is possible to directly compare changes in gene expression, not only in different tissues of the same species, but also in similar tissues among different species. We investigated whether a transcriptomic approach using ovariectomized rats would be an adequate model to predict (post)menopausal health risks and/or benefits of soy isoflavone supplementation. Therefore, in the rat study reported here we have investigated the changes in gene expression in different target (i.e. breast, uterus and sternum tissues) and non-target (PBMC, adipose and liver tissues) tissues of ovariectomized female F344 rats exposed to a commercially available soy supplement for eight weeks in an oral dose which, on a kg bw base, is similar as the recommended dose for humans. In addition, the changes in gene expression in PBMC in the current study were compared with those of a previously reported short-term rat study [18]. Finally a cross-species comparison of changes in gene expression in PBMC and adipose tissues among ovariectomized rats and (post)menopausal women was carried out to compare the effects of soy isoflavone supplementation in rats with those observed in (post)menopausal human volunteers. The overall aim of the present study was to investigate whether such a toxicogenomic approach could be used to predict possible health effects in humans, and would improve the health risk/benefit assessment of soy isoflavones.

2. Materials and methods

2.1. Chemicals

Pure isoflavone standards (both glucosides and aglycones) and equol were purchased from LC laboratories (Woburn, MA, USA). Dimethyl sulfoxide (DMSO) was obtained from Acros Organics (Geel, Belgium), and enterodiol, β-glucuronidase H-5 (*H. Pomatia* contain 60,620 unit/g solid), Optiprep™, and Tricine from Sigma-Aldrich (Steinheim, Germany). NaCl was obtained from VWR International (Darmstadt, Germany), and acetonitrile and methanol from Biosolve BV (Valkenswaard, the Netherlands). Oasis HLB 1cc solid phase extraction cartridges were obtained from Waters (Milford, MA, USA).

2.2. Animals

Twelve female ovariectomized F344 inbred 8 weeks old rats were purchased from Harlan, (Horst, the Netherlands). The ovariectomy of these rats was done at the age of 6 weeks by the supplier and after 2 weeks of acclimatization they were delivered at the animal facility, the Centre for Laboratory Animals (CKP), Wageningen University, Wageningen, the Netherlands. Six rats were used as control and six were used for soy isoflavone treatment. F344 rats were chosen for several reasons. Firstly, according to US EPA (Environmental Protection Agency) [20], the inbred isogenic strain F344 rats are sensitive to estrogenic compounds and thus particularly suitable to study the effects of soy isoflavones. Secondly, the use of an inbred strain will minimize the background noise in micro array analysis. Thirdly, for reasons of comparison, we used the same strain of rats as for the short-term study and measured PBMC gene expression; this enabled comparison of PBMC gene expression after long term exposure (present study) and short term exposure [18].

2.3. Housing and diet

After arrival at the animal facility (i.e. CKP), the rats were housed in groups (2 animal/cage) and fed an isoflavone free RMH-B standard diet (ABDiets, Woerden, the Netherlands) (Supplementary Table 1) ad libitum. Standard housing, day-light hours and humidity were also maintained during the whole experimental period. The experiment started after a week acclimatization period. After that period animals were about 9 weeks of age with an average body weight of 160.8 ± 9.8 g.

2.4. Animal experiment

All aspects of the experimental protocol were reviewed and approved by the Animal Welfare Committee of Wageningen University (Wageningen, the Netherlands) which was in compliance with the Dutch Act on animal experiment (Stb, 1977, 67; Stb 1996, 565), revised February 5, 1997. The approval number was 2013015.d. The animals were dosed a commercial soy containing supplement, corresponding to a dose of 2 mg soy isoflavones/kg bw (1 gavage/day) in 1% DMSO solution for seven days per week, during 8 weeks. One day before sacrifice the animals received an extra dose in the evening instead of a dose early next morning. This was done to make the experiment comparable with the time schedule of the two human intervention studies (see below) carried out by the Division of Human Nutrition, Wageningen University in order to facilitate direct comparison of both rat and human data after exposure to the same supplement. The dose level used (i.e. 2 mg soy isoflavones/kg body weight per day) provides the equivalent dose for a female adult consuming the same soy based supplement. This dose was also selected to provide a direct comparison with the human intervention studies (mentioned in Section 2.5).

2.5. Related experiments

The effects on gene expression in PBMC and adipose tissue after soy isoflavones intake in the present rat study were compared with the gene expression data of a previously reported short term rat study, and of two human intervention studies (see below).

2.5.1. Short term rat study

A short-term (2 days) rat experiment was performed earlier at the same animal facility centre of Wageningen University, after approval by the ethical committee on animal experimentation of Wageningen University. This short-term study, in which rats from the same strain (i.e. ovariectomized F344) were exposed to a similar soy isoflavone supplement, and also PBMC were collected and studied by microarray analysis, was mainly designed for quantification of the bioavailability of soy isoflavone [18].

2.5.2. Human intervention studies with the same commercial soy supplement

Two human intervention studies were conducted at the Division of Human Nutrition of Wageningen University [4,19]. The studies were approved by the Medical Ethical Committee of this university and registered at clinicaltrials.gov under number NCT01232751 and NCT01556737. Both of these studies were double blind cross-over studies with the exposure time of two eight week intervention periods and an eight week washout period in between. A total of 30 (post)

menopausal, equol-producing women with an average age of 61.1 ± 5.8 years were recruited for the first human intervention study in which PBMCs were collected [4]. Twenty six (post)menopausal women were recruited for the second human intervention study with the same supplement as the first study and in which adipose tissue was collected [19]. All volunteers were recruited from Wageningen or the direct surrounding areas. All participants received the same soy supplement as used in the rat studies and 2 capsules in the morning and 2 in the evening after the meal resulting in a dose of about 1.5 mg soy isoflavones/kg bw per day and a placebo treatment.

2.6. Preparation of test solutions

The commercial soy supplement, Phytosoya, was obtained from Arkopharma (Carros, France). The supplement had a total soy isoflavones content of 70.3 mg/gram supplement [21]. The content of the three glucosides; genistin, daidzin and glycitin was 7.54, 40.03 and 22.72 mg/g supplement, respectively. An oral gavage dose of 2 mg soy isoflavones/kg bw which corresponds with 0.4 mg soy isoflavones/rat was applied per day. The stock solutions were freshly prepared daily, sonicated for 30 min and were placed on a multi-axile-rotating-mixture (CAT RM-40, Laufen, Germany) machine to avoid any aggregation during the period of gavage dosing. The control rats received a gavage dose of nanopure water containing 1% DMSO.

2.7. Sample collection and analysis

After the 8 weeks dosing the animals were sacrificed after anaesthesia with a mixture of isoflurane and oxygen followed by removal of blood from the dorsal aorta with a syringe containing 0.1 mL lithium heparin. The collected blood was immediately transferred into 10 mL tubes and kept on a multi-axle-rotating mixer (CAT RM-40, Laufen, Germany). The average amount of collected blood was 5–6 mL. Collected blood samples were divided into two parts, one part (3.5 mL) for isolating PBMC and another part (about 1–2 mL) for separating plasma. The first part of the collected blood samples was used for micro array analysis and the second part was used for measuring the plasma concentration of soy isoflavone. White adipose, breast, uterus, liver, and sternum tissues were also collected. After collection the tissue samples were frozen immediately in liquid nitrogen and stored at −80 °C until further analysis. The plasma samples were prepared by centrifuging the blood for 5 min at 10,000 rpm (Biofuge centrifuge, Heraeus Sepatech, UK) and were collected in Safe-lock 0.5 mL Eppendorf tubes (Eppendorf AG, Hamburg, Germany). These samples were placed on ice during the sample collection of the in vivo experiment and stored at −80 °C until analysis.

2.8. Extraction and analysis of soy isoflavones from rat plasma

The enzymatic hydrolysis, solid phase extraction and HPLC (High Performance Liquid Chromatography) analysis of soy isoflavone in plasma samples were performed as described earlier [19,21]. To avoid inter laboratory variations among the present in vivo rat study and the human intervention studies, analysis of soy isoflavones concentrations in plasma and micro-array analysis were performed at the same laboratories using similar protocols. Averages and standard deviations of plasma soy isoflavones concentrations of the dose group and the control were calculated using Microsoft Excel.

2.9. Isolation of rat PBMC, mRNA and running microarray analysis of different tissues

Packages from the Bioconductor project [22], integrated in an online software program [23], were used to analyse the array data. Various advanced-quality metrics, diagnostic plots, pseudo images, and classification methods were used as described by Heber and Sick [24], to determine the quality of the arrays before statistical analysis. The probes on the Rat Gene 1.1 ST array were redefined using current genome information [25]. In this study, probes were reorganized on the basis of the gene definitions available in the National Center for Biotechnology Information (NCBI) *Rattus norvegicus* Entrez Gene database, based on the rat genome build 5.1 (custom CDF v17). Normalized gene expression estimates were calculated with the Robust Multichip Average method [26]. Subsequently, the dataset was filtered to only include probe sets that were active (i.e. expressed) in at least 4 samples, using the universal expression code (UPC) approach (UPC score > 0.50) [27]. Differentially expressed probe sets were identified by using linear models, applying moderated t-statistics that implemented intensity-based empirical Bayes regularization of standard errors [28,29]. Probe sets with $p < 0.05$ were considered to be significantly regulated. Changes in gene expression were related to functional changes using gene set enrichment analysis (GSEA) performed according to Subramanian et al. [30]. GSEA focuses on groups of genes that share a common biological, biochemical or metabolic function. GSEA has the advantage that it is unbiased, because no gene selection step is used. Only gene sets consisting of more than 15 and less than 500 genes were taken into account. Gene sets were derived from the Kyoto Encyclopedia of Genes and Genomes (KEGG) [31]. Effects of soy isoflavones treatment versus control were compared using ranked lists based on significance (t-values), using methods described by Plaisier et al. [32].

3. Results

Fig. 1 shows an overview of the results from the microarray analysis of ovariectomized female F344 rats. After normalization and filtering of a total of 19,311 probe sets, 39–50% probe sets were retained in the different tissues. The percentage of significantly regulated ($p < 0.05$) genes within this data set amounted to only 1–13.6% (i.e. 74 to 1305 genes out of 7556 to 9561 robust multichip average (RMA) normalized and UPC filtered genes), and was lowest for the breast tissue (i.e. only 1%), with increasing number of genes being regulated in sternum, PBMC, liver, adipose and uterus tissues (i.e. 2.3, 6.1, 10.4, 13.6 and 13.6%, respectively). Fig. 1 also shows the number of significantly up and down regulated (false discovery rate, FDR < 0.25) biological pathways. In line with the total number of significantly regulated genes, the lowest number of significantly changed biological pathways was found in breast tissue (i.e. 68 gene sets) and the highest number in uterus tissue (i.e. 309 gene sets). In the right column of Fig. 1 the human homologous genes in PBMC and adipose tissue which were also found in the rat are indicated. These homologous genes were retrieved from the human intervention studies mentioned above [4,19]. Out of 6413 and 7731 human homologous genes, only 10 and 88 genes were significantly ($p < 0.05$) regulated in rat PBMC and adipose tissue, respectively.

As the effects of soy isoflvones are considered to be mostly mediated via the estrogen receptors (ERs), we investigated the number of differentially regulated ERGs in the six selected rat tissues. These genes were selected based on their occurrence in the Dragon DRGs database (http://datam.i2r.a-star.edu.sg/ergdbV2/). Table 1 shows the list of ERGs and the direction of their regulation in the different rat tissues. It is shown that out of 87 ERGs available in the specified website only 19 were found to be significantly regulated in the different tissues. The highest number of ERGs that were significantly regulated was found in liver tissue (7 ERGs) followed by adipose tissue (6 ERGs), uterus tissue (5 ERGs) and PBMC (3 ERGs). Surprisingly no ERGs were significantly regulated in breast and sternum tissues. Moreover, it appeared that there was no similarity in the pattern of regulation of the various ERGs among the different tissues.

Because most of the ERGs were not or only slightly regulated by soy isoflavone administration in different tissues of ovariectomized rat, we investigated the genes that were affected most in the three target

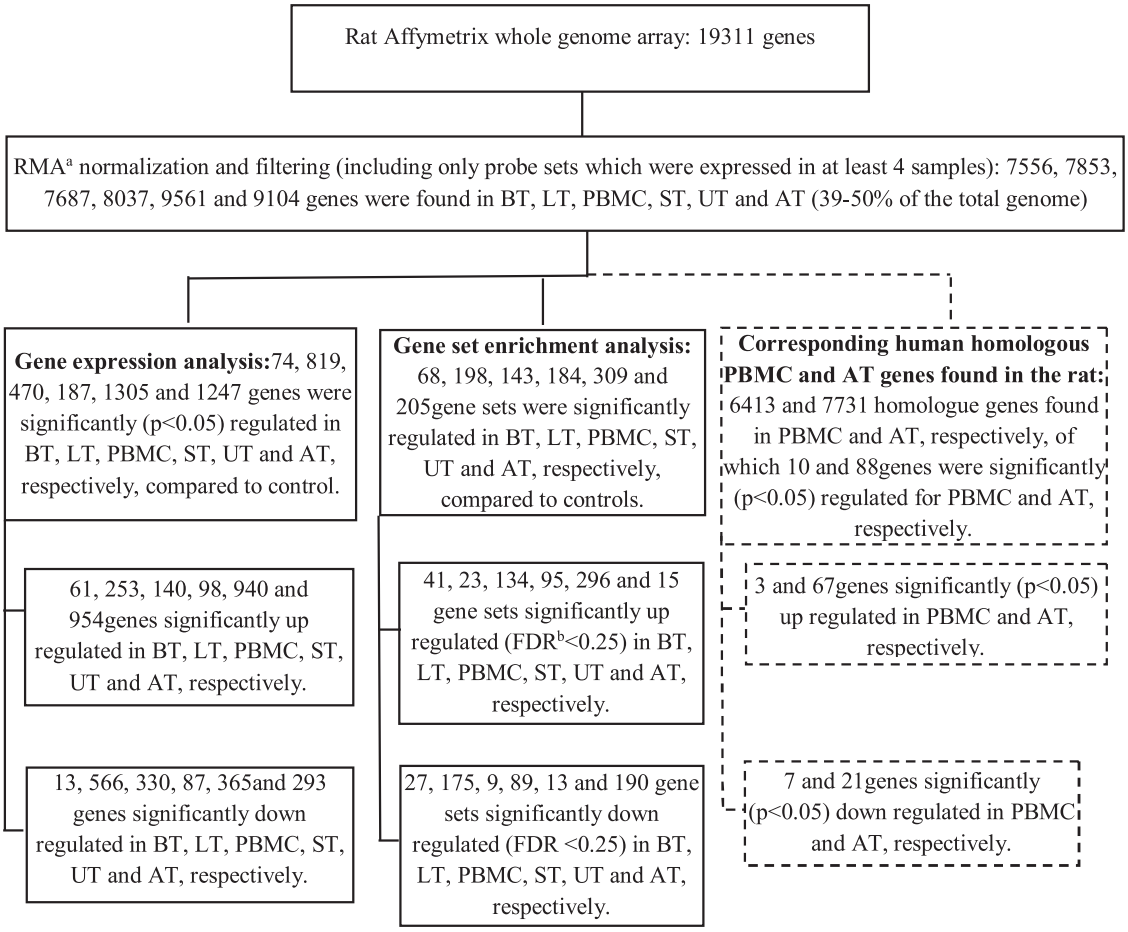

Fig. 1. Flow chart of the microarray analysis indicating significantly changed genes and gene sets induced in different tissues of ovariectomizedF344 rats after oral gavage dosing (2 mg soy isoflavones/kg bw per day for 8 weeks) of a commercial soy supplement. The number of corresponding human homologue genes found in rats is also indicated (right hand column). AT, adipose tissue; BT, breast tissue; LT, liver tissue; PBMC, peripheral blood mononuclear cells; ST, sternum tissue and UT, uterus tissue.

tissues, namely breast, uterus and sternum, by using a 'fold change (FC)' threshold. Only significantly affected genes with an FC value > 2 were selected to be investigated for their involvement in different biological processes, and the higher the FC value the more the gene is affected. Table 2 shows the significantly regulated genes, with an FC > 2. In ST, no genes were regulated with an FC value > 2. In breast tissue 14 genes with an FC > 2 were identified (i.e. 19% of the significantly regulated genes) of which 13 were up regulated, and in uterus tissue 11 genes (i.e. 1% of the significantly regulated genes) of which 9 were up regulated. According to the information in the NCBI database the up regulated genes are involved in metabolism such as fatty acid, lipid and glucose metabolism, in stress responses such as detection of oxidative stress, responses to abiotic stimuli, defence mechanisms and immune responses such as cellular response to tumour necrosis factor, antigen binding, and intercellular signal transduction. Down regulated genes are involved in DNA and protein binding and inflammatory response.

Table 3 shows the significantly regulated ($p < 0.05$) biological pathways (BPs) in different tissues of the present rat study, and the related human disease, as indicated in the Kyoto Encyclopaedia of Genes and Genomes (KEGG) data base. The total list of biological pathways that were significantly up- or down regulated in different tissues can be found in supplementary Table 2. A total of 33 diseases related KEGG based biological pathways were found to be significantly regulated in different tissues in the present rat study. The highest number of up regulated biological pathways was found in uterus tissue (i.e. 24) and then in breast tissue (i.e. 5), whereas 3 biological pathways were up regulated in sternum and PBMC. The highest number of down regulated biological pathways was found in liver tissue (i.e. 19) followed by adipose tissue (10), PBMC (7), and uterus tissue (2). Although only 5 and 3 biological pathways were significantly regulated in breast and sternum tissue, respectively, they all were up regulated, whereas in liver and adipose all the BPs were down regulated. Interestingly, when a disease related pathway was significantly up regulated in the surrogate tissues, PBMC or adipose tissue, it was mostly down regulated in one or more target tissues, and vice versa. No direct correlation was found between effects in the surrogate tissues and the target tissues. Also in the gene set enrichment scatter graphs we made to compare up and down regulated BPs in different surrogate and target tissues (results not shown), not any correlation was observed.

In Fig. 2 the gene expression patterns in PBMC of the present rat study were compared to those obtained in the short-term rat study [18]. Only 145 genes were significantly regulated in both data sets (data not shown) of which fifteen marker genes were identified to be highly correlated (deviation value 0 to ± 10) and their gene expression was significantly changed in the same direction (increased or decreased) in both studies. As can be concluded from the information included in supplementary Table 3, most of the genes thus identified are involved in biological pathways related to increase in immune response and in cell proliferation.

In the human intervention studies mentioned above, van der Velpen et al. [4,19] reported significant regulation of ERGs in human PBMC and adipose tissue following soy isoflavone supplementation. Table 4 shows the reported significantly expressed ERGs in PBMC of human volunteers that were also significantly regulated in PBMC of the present

Table 1
List of significantly ($p < 0.05$) up and down regulated estrogen-responsive genes (ERGs) in different tissues of ovariectomized rats after 8 weeks oral dosing with the frequency of one gavage dose/day of a commercial supplement (2 mg soy isoflavones/kg bw) compared with control. Up and down regulated enrichments were indicated by up and down arrow marks. A list of all ERGs can found in the Dragon ERG database (https://web.archive.org/web/20160118215946/http://datam.i2r.a-star.edu.sg/ergdbV2).

Name of the gene	PBMC	AT	BT	UT	ST	LT
AR	↓	↑ns	↓ns	↓ns	↓ns	absent
ANXA4	↓ns	↓ns	↑ns	↑ns	↓ns	↓
DUSP1	↓ns	↓ns	↓ns	↓ns	↓ns	↓
IGF1	absent	↑ns	↑ns	↑ns	↑ns	↓
IL6R	↓ns	↑ns	↑ns	↑	↑ns	↑
IGFBP5	absent	↓ *	↑ns	↓ns	↑ns	absent
IGFBP2	absent	↑ *	absent	absent	absent	↓ns
KIT	absent	↑ *	↑ns	absent	absent	absent
NR2F6	absent	↓ *	absent	↑ns	absent	↓ns
PGR	absent	absent	absent	↑	absent	absent
PNOC	absent	↑	absent	↓	absent	absent
PAWR	absent	↑ *	↓ns	↓ns	absent	↓ns
RARA	↓ *	↓ns	↓ns	↑ns	↓ns	↓
RB1	↑ns	↑ns	↑ns	↑ns	↓ns	↓
SCARB1	↓ns	↑ns	↑ns	↑	↑ns	↑ns
SCP2	↑ns	↑ *	↑ns	↓ns	↑ns	↑ *
TGFB1	↓ns	↓ns	↑ns	↑	↑ns	↓ns
TEP1	↓ *	↓ns	↓ns	↑ns	↑ns	↓ns
TIMP1	absent	↑ns	↑ns	↑	↑ns	↓ns

ns = not significant.
* genes common in rat and human.
AT, adipose tissue; BT, breast tissue; LT, liver tissue; PBMC, peripheral blood mononuclear cells; ST, sternum tissue and UT, uterus tissue.

rat study. Out of 17 significantly changed human PBMC genes, only 1 gene, namely CACYBP, was significantly regulated in the rat, although in opposite direction (up in humans and down in rats), 5 genes were insignificantly regulated, and 11 were not differentially regulated in rat PBMC at all. In summary, no correlation was found between the effects on gene regulation in PBMC of the present rat study and those in PBMC of the study in human volunteers.

Table 5 shows the reported significantly expressed ERGs in adipose tissue of human volunteers [4] and their expression in the adipose tissue in the present rat study. Out of 82 significantly changed ERGs in human adipose tissue, only 6 genes were also significantly changed in rat adipose tissue, 40 genes were non-significantly regulated and 36 genes were not regulated in the rat adipose tissue. Out of the 6 genes that were significantly regulated in both human and rat adipose tissue, 5 were regulated in the same direction (up or down), and 1 (KITLG) changed in the opposite direction. The down regulated common ERGs in adipose tissue i.e. NR4A1 and IGF2 have a positive relationship with different BPs such as apoptosis, endothelial cell proliferation, RNA and DNA transcription, T-cell proliferation etc. (see supplementary Table 4). Thus down regulation of these genes would lead to inhibition of these processes. Although some similarities in the response in gene expression in rat and human adipose tissue following soy isoflavone exposure have been found, there is no clear overall correlation.

Fig. 3 shows a rank-rank scattered plot of the significantly regulated genes in PBMC and adipose tissue in the rat study and in the human intervention studies. There were 10 human homologous genes significantly regulated in rat PBMC, however there was no overall correlation between these 10 genes and the 10 significantly regulated human PBMC genes (Fig. 3a). This implies that the genes which were highest up regulated in rat PBMC were not the same as those in human PBMC, and some genes were regulated in the opposite direction. A similar result was found for the 88 common and significantly regulated genes expressed in rat and human adipose tissue (Fig. 3b). To identify marker genes, i.e. genes of which the expression was increased or decreased in the same direction in both rat and human PBMC and adipose tissue from the rank-rank scattered plots shown in Fig. 3 a and b, deviation values were used from the corresponding correlation line. For PBMC (Fig. 3a) a deviation value of 0 to ± 1 was used, because the number of overlapping genes was only 10. For adipose tissue (Fig. 3b) a deviation value of 0 to ± 4 was used, because the number of overlapping genes was 88. For PBMC (Fig. 3a) three marker genes were identified, which were mainly associated with protein production and regulation of cell growth (see supplementary Table 5). Similarly 11 marker genes were

Table 2
List of the significantly expressed (fold change > 2 and $p < 0.05$) genes in different target tissues of rats (negative numbers indicate down regulation).

Tissue	Gene symbol	Description	Functions/process	Fold change
Breast tissue	Adipoq	Adiponectin, C1Q and collagen domain containing	Fat cell differentiation, detection of oxidative stress, fatty acid oxidation and glucose metabolism.	4.9
	Rnase2	Ribonuclease, RNase A family, 2 (liver, eosinophil-derived neurotoxin)	A protein-coding gene involved in nucleic acid binding.	4.5
	Mcpt1l1	Mast cell protease 1-like 1	Involved in proteolysis (protein breakdown).	2.9
	Cpa3	Carboxypeptidase A3, mast cell	A protein-coding gene involved in zinc ion binding	2.9
	Thrsp	Thyroid hormone responsive	Lipid metabolism biosynthesis process.	2.8
	Cd163	CD163 molecule	Receptor mediated endocytosis	2.3
	Tusc5	Tumour suppressor candidate 5	Involved in the response to the biotic stimulus	2.3
	Cdo1	Cysteine dioxygenase type 1	Metabolism of cysteine	2.3
	Fabp4	Fatty acid binding protein 4, adipocyte	Fat cell differentiation, cholesterol homeostasis, fatty acid metabolism and cytokine production	2.2
	Prkar2b	Protein kinase, cAMP dependent regulatory, type II bet	Fatty acid metabolism	2.1
	Mpz	Myelin protein zero	Cell-cell junction maintenance	2.1
	Atf3	Activating transcription factor 3	Involved in DNA and protein binding,	−2.1
Uterus tissue	LOC684146	Ig kappa chain V-II region 26-10-like	No information provided by NCBI database	10.2
	Igkv28	Immunoglobulin kappa chain variable 28	No information provided by NCBI database	8.7
	LOC500181	Ig kappa chain V-V region K2-like	No information provided by NCBI database	3.4
	Igj	Immunoglobulin joining chain	A protein-coding gene involved in antigen binding	2.9
	Nos2	Nitric Oxide Synthase 2, Inducible	Involved in aging, cellular response to tumour necrosis factor, intracellular signal transduction and nitric acid biosynthetic process	2.8
	Mzb1	Marginal zone B and B1 cell-specific protein	Involved in integrin activation, regulation of B cell proliferation and positive regulation of immunoglobulin biosynthetic process	2.7
	LOC100361706	Lambda-chain C1-region-like	Involved in antigen binding	2.5
	LOC362795	Immunoglobulin G heavy chain	Involved in antigen binding	2.2
	Fgg	Fibrinogen gamma chain	Involved in inflammatory response, platelet activation and aggregation	−2.1
	Rup2	Urinary protein 2	No information provided by NCBI database	−3.9

Table 3
Significantly (p value < 0.05) regulated biological pathways after 8 weeks oral administration of a commercial soy supplement in ovariectomized rats. Only KEGG based biological pathways were included that were related to a distinct human disease. Up and down regulation is indicated by up and down arrows.

KEGG BASES BPs	PBMC	AT	LT	BT	ST	UT
Alzheimer's disease	↑	↓	ns	ns	ns	ns
Parkinson's disease	↑	↓	ns	ns	ns	↓
Huntington's disease	↑	↓	ns	ns	ns	ns
Prion diseases	ns	↓	↓	ns	ns	ns
Bacterial invasion of epithelial cells	ns	↓	↓	↑	ns	↑
Leishmaniasis	↓	↓	↓	ns	ns	↑
Chagas disease (americantrypanosomiasis)	ns	ns	ns	ns	↑	↑
African trypanosomiasis	absent	absent	ns	ns	ns	↑
Malaria	ns	ns	↓	↑	↑	↑
Toxoplasmosis	ns	ns	↓	ns	ns	↑
Amoebiasis	ns	ns	↓	ns	↑	↑
Staphylococcus aureus infection	↓	ns	↓	↑	ns	↑
Hepatitis c	ns	ns	ns	ns	ns	↑
Pathways in cancer	ns	ns	↓	ns	ns	↑
Pancreatic cancer	ns	ns	↓	ns	ns	↑
Glioma	ns	ns	↓	ns	ns	↑
Thyroid cancer	ns	ns	ns	ns	ns	↑
Melanoma	ns	ns	↓	ns	ns	ns
Bladder cancer	ns	ns	↓	ns	ns	ns
Chronic myeloid leukemia	ns	ns	ns	ns	ns	↑
Acute myeloid leukemia	↓	ns	ns	ns	ns	↑
Small cell lung cancer	ns	ns	↓	ns	ns	↑
Non-small cell lung cancer	ns	ns	ns	ns	ns	↑
Autoimmune thyroid disease	↓	ns	ns	ns	ns	↑
Systemic lupus erythematosus	ns	ns	↓	↑	ns	↑
Rheumatoid arthritis	ns	↓	↓	↑	ns	↑
Allograft rejection	↓	absent	ns	ns	ns	↑
Graft-versus-host disease	↓	absent	ns	ns	ns	↑
Primary immunodeficiency	↓	absent	ns	ns	ns	↑
Hypertrophic cardiomyopathy (hcm)	ns	↓	↓	ns	ns	↓
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	ns	↓	↓	ns	ns	ns
Dilated cardiomyopathy	ns	↓	↓	ns	ns	ns
Viral myocarditis	ns	↓	↓	ns	ns	↑

ns = not significant.
BPs, biological pathways; AT, adipose tissue; BT, breast tissue; LT, liver tissue; PBMC, peripheral blood mononuclear cells; ST, sternum tissue and UT, uterus tissue.

identified for adipose tissue (Fig. 3b). As can be seen in the supplementary table 6, these marker genes appeared to be involved in biological processes related to immune and inflammatory responses. In addition scattered plots were made using all homologous genes (i.e. 6413 homologous genes for human and rat PBMC and 7731 homologous genes for human and rat adipose tissue, see Fig. 1) without applying any significant cut-off value, but also in this situation no correlation between results in rats and humans was observed (results not shown).

4. Discussion

The results of this eight week study in ovariectomized rats show that the overall gene expression data after soy isoflavone supplementation are tissue specific for the regulation of estrogen responsive genes (ERGs) (see Table 1), and even more for genes not known to be ERGs. The different responses in gene expression in different tissues might be explained different ERα/ERβ ratios in these tissues [11–14]. This may complicate the use of surrogate tissues like PBMC or adipose tissue to predict the effects in target tissues such as breast, uterus or sternum tissue. In addition to differences in gene regulation upon soy isoflavone supplementation among different tissues, there also appeared to be a considerable difference in gene expression in the same tissue of the same species upon different exposure duration, as was evident by the absence of a correlation between the gene expression data in PBMC

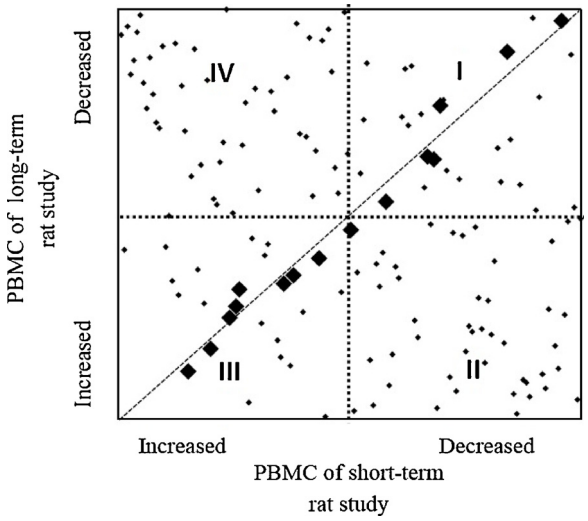

Fig. 2. Rank-rank scattered plot for significantly changed common PBMC genes in the current long-term rat study (i.e. 8 week exposure) and from the group exposed to the same dose (i.e. 2 mg soy isoflavones/kg bw per day) in the short-term (2 days) rat study [18]. Each dot represents the t-value of a single gene and the highlighted dots (♦) indicate the highly correlated genes. The list of these 15 genes can be found in supplementary Fig. 3. Genes significantly changed in the same direction in both treatments are in Cartesian quadrants I and III, while genes significantly changed in opposite directions are in quadrants II and IV.

Table 4
List of the significantly expressed human estrogen responsive genes (ERGs) in PBMC of human volunteers reported by van der Velpen et al. [19] and their expression in rat PBMC in the present long-term rat study. Up and down regulated gene expressions are indicated by up and down arrows.

Genes	Description	Regulation	
		Human	Rat
BCL2L1	BCL2-like 1	↑	a
CACYBP	Calcyclin binding protein Y	↓	↑
EDEM1	Endoplasmatic reticulum degradation enhancer, mannosidase alpha-like 1	↑	↑ns
ERBB2	V-ERB-B2 erythroblasticleukemia viral oncogene homolog 2 neuro/glioblastoma derived oncogene homolog (avian)	↑	a
FKBP5	FK506 binding protein 5	↑	a
FOXP1	Forkhead box P1	↑	↓ns
HSPA1 A	Heat shock 70 kDa protein 1 A Y	↓	a
MYB	v-mybmyeloblastosis viral oncogene homolog (avian)	↑	a
NME2	NME/NM23 nucleoside diphosphate kinase 2 Y	↓	↑ns
NRP1	Neuropilin 1 Y	↓	↓ns
PSMD8	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 8 Y	↓	↑ns
PTPRO	Protein tyrosine phosphatase, receptor type, O Y	↓	a
SLC25A5	Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5	↓	a
SPRED1	Sprouty-related, EVH1 domain containing 1 Y	↓	a
STAB1	Stabilin 1 Y	↓	a
STXBP1	Syntaxin binding protein 1	↑	a
TIMELESS	Timeless homolog (Drosophila)	↓	a

a = absent.
ns = not significant.

collected in the short- and the long-term rat study (see Fig. 2). We also did not observe a correlation between changes in gene expression in similar tissues (PBMC and adipose tissue) from rats and humans upon similar dosing and exposure duration (see Fig. 3; Table 4 and 5).

It was the aim of the present study to investigate whether it was possible to predict the effect of soy isoflavones supplementation in human target tissues, such as breast, uterus or sternum tissue. This was

Table 5
Significantly expressed human estrogen responsive genes (ERGs) in adipose tissue of human volunteers reported by van der Velpen et al. [4] and their expression in adipose tissue in the present long-term rat study. Up and down regulated gene expressions are indicated by up and down arrows.

Sl. No.	Genes	Description	Regulation	
			Human	Rat
1	NR4A1	nuclear receptor subfamily 4, group A, member 1	↓	↓
2	RET	ret proto-oncogene	↓	a
3	TK1	thymidine kinase 1, soluble	↓	a
4	TGFB3	transforming growth factor, beta 3	↓	↑ns
5	NOS3	nitric oxide synthase 3, endothelial cell	↓	↓ns
6	GOT1	glutamic-oxaloacetic transaminase 1, soluble	↓	↓ns
7	TNC	tenascin C	↓	a
8	IGF2	insulin-like growth factor 2	↓	↓
9	PPIF	peptidylprolylisomerase F	↓	↑ns
10	ME1	malic enzyme 1, NADP(+)-dependent, cytosolic	↓	↓ns
11	NME1	NME/NM23 nucleoside diphosphate kinase 1	↓	a
12	TUBG1	tubulin, gamma 1	↓	↑ns
13	THBD	Thrombomodulin	↓	a
14	NCAM2	neural cell adhesion molecule 2	↓	a
15	KITLG	KIT ligand	↓	↑
16	ELOVL2	ELOVL fatty acid elongase 2	↓	a
17	CCND1	cyclin D1	↓	↑ns
18	CNKSR3	CNKSR family member 3	↓	a
19	CYCS	cytochrome c, somatic	↓	a
20	DHCR24	24-dehydrocholesterol reductase	↓	↓ns
21	KIR3DL2	killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail, 2	↓	a
22	PAICS	phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazolesuccinocarboxami	↓	a
23	TFF1	trefoil factor 1	↓	a
24	HSPD1	heat shock protein 1 (chaperonin)	↓	↑ns
25	KPNA2	karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	↓	a
26	ARMCX3	armadillo repeat containing, X-linked 3	↓	↑ns
27	ENO1	enolase 1, (alpha)	↓	↑ns
28	SGCD	sarcoglycan, delta (dystrophin-associated glycoprotein)	↓	↓ns
29	SEMA5B	sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembranedomai	↓	a
30	G6PD	glucose-6-phosphate dehydrogenase	↓	↑ns
31	RAMP3	receptor (G protein-coupled) activity modifying protein 3	↓	a
32	AURKB	aurora kinase B	↓	a
33	ESR2	estrogen receptor 2 (ER beta)	↓	a
34	C1QBP	complement component 1, q subcomponent binding protein	↓	↑ns
35	CENPA	centromere protein A	↓	a
36	ACO2	aconitase 2, mitochondrial	↓	↑ns
37	RUNX1	runt-related transcription factor 1	↓	↓
38	MCM4	minichromosome maintenance complex component 4	↓	a
39	NR4A3	nuclear receptor subfamily 4, group A, number 3	↓	a
40	FOXF1	forkbead box F1	↓	a
41	ORMDL2	ORM1-like 2 (S. cerevisiae)	↓	↑ns
42	MARCKS	myristoylated alanine rich protein kinase C substrate	↓	↑ns
43	SLC12A2	solute carrier family 12 (sodium/potassium/chloride transporter), member 2	↓	↑ns
44	SPRY1	sprout homolog 1, antagonist of FGF signaling (Drosophila)	↓	a
45	GARS	glycyl-tRNAsynthetase	↓	↓ns
46	RPA3	replication protein A3	↓	↑ns
47	STMN1	stathmin 1	↓	a
48	IARS	isoleucyl-tRNAsynthetase	↓	↓ns
49	ITGAV	integrin, alpha V	↓	↑ns
50	NUP88	nucleoporin 88	↓	↑ns
51	TXNIP	thioredoxin interacting protein	↑	↑ns
52	NRF1	nuclear respiratory factor 1	↑	↑ns
53	ECE1	endothelin converting enzyme 1	↑	↑ns
54	EFEMP1	EGF-containing fibulin-like extracellular matrix protein 1	↑	↑
55	MPL	Myelo-proliferative leukemia virus oncogene	↑	a
56	GSTO1	glutathione S-transferase omega 1	↑	↑ns
57	HIP1R	huntingtin interacting protein 1 related	↑	a
58	PAX8	paired box 8	↑	a
59	PTPN18	protein tyrosine phosphatase, non-receptor type 18 (brain-derived)	↑	a
60	WSB1	WD repeat and SOCS box-containing 1	↑	↑ns
61	IGF1R	insulin-like growth factor 1 receptor	↑	↑ns
62	GNG7	guanine nucleotide binding protein (G protein), gamma 7	↑	a
63	SFRP1	secreted frizzled-related protein 1	↑	↑ns
64	MCM7	minichromosome maintenance complex component 7	↑	↑ns
65	SATB1	SATB homeobox 1	↑	↓ns
66	INPP4B	inositol polyphosphate-4-phosphatase, type II	↑	↓ns
67	THBS2	thrombospondin 2	↑	↑ns
68	PTGER2	prostaglandin E receptor 2 (subtype EP2), 53kDa	↑	a
69	WISP2	WNT1 inducible signaling pathway protein 2	↑	↑
70	BCL2L11	BCL2-like 11 (apoptosis facilitator)	↑	a
71	ABCB1	ATP-binding cassette, sub-family B (MDR/TAP), member 1	↑	a

(*continued on next page*)

Table 5 (*continued*)

Sl. No.	Genes	Description	Regulation	
			Human	Rat
72	TSC22D3	TSC22 domain family, member 3	↑	a
73	NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	↑	↑ns
74	ANKRD44	ankyrin repeat domain 44	↑	↑ns
75	CTNND1	catenin (cadherin associated protein), delta 1	↑	↑ns
76	BCL2L1	BCL2-like 1	↑	a
77	TNFSF8	tumour necrosis factor (ligand) superfamily, member 8	↑	a
78	CCNG2	cyclin G2	↑	↑ns
79	FKBP8	FK506 binding protein 8	↑	↓ns
80	ZFP36L2	zinc finger protein 36, C3H type-like2	↑	a
81	SLA	Src-like adaptor	↑	↑ns
82	S100P	S100 calcium binding protein P	↑	a

a = absent.
ns = not sigsssssssnificant.

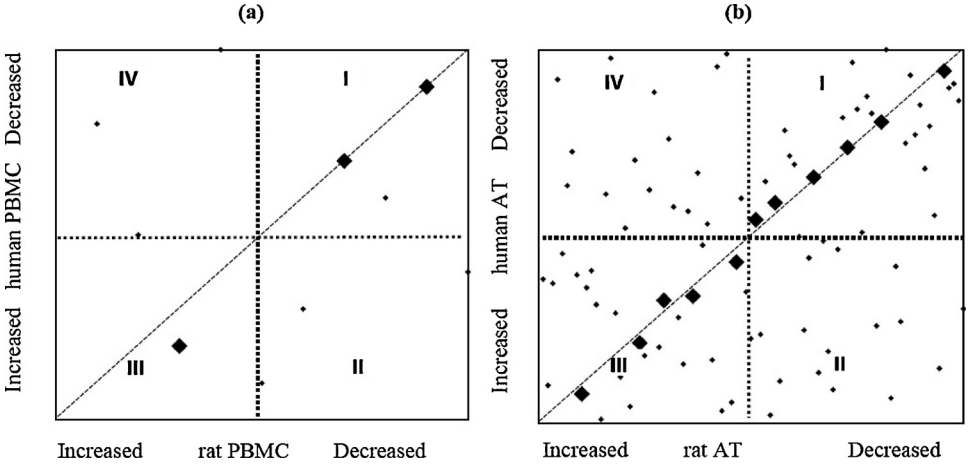

Fig. 3. Rank-rank scattered plot for significantly changed common PBMC genes in rat and (post)menopausal women (3a), and significantly changed common genes in adipose tissue of rats and (post)menopausal women (3b). Each dot represents the t-value of a single gene and the highlighted dots (♦) indicate the correlated genes. Corresponding human homologous genes were retrieved from the study with (post)menopausal women taking the same supplement, in a similar dose (about 1.5 mg soy isoflaone/kg bw per day) and time duration (i.e. 8 weeks) [4,19]. Genes significantly changed in the same direction in both treatments are in Cartesian quadrants I and III, while genes significantly changed in opposite directions are in quadrants II and IV.

done by evaluating the similarities in the gene expression pattern of the surrogate tissues PBMC and adipose tissue in rats and humans after administration of the similar oral dose of a commercially available soy isoflavone containing supplement. Because the results of the surrogate tissues (PBMC and adipose tissue) in rats differed from those in humans, and because there was no correlation between changes in gene expression between surrogate and target tissues in rats, we were unfortunately not able to predict possible health effects in humans. Although transcriptomic techniques are a powerful tool to predict early biological effects, it appeared to be complicated to interpret early changes in gene expression profiles in relation to adverse or beneficial responses related to different biological processes.

In our earlier short term rat experiment [18] we have found that after exposure of ovariectomized rats for 2 days to the same supplement as used in the present study, 3861 genes were significantly regulated in PBMC. In the present 8 weeks study the number of regulated genes in PBMC was significantly smaller (470 genes). Compared with the total number of probes in the microarray, the number of significantly regulated genes in the short term study was 20% and in the long term study only 2.4%. Interestingly, in the human intervention study reported by van der Velpen et al. [19] it was found that 357 genes were significantly regulated in human PBMC, which was about 1.8% of the total probe sets. Possibly long-term exposure might induce homeostasis, resulting in a smaller number of regulated genes in PBMC gene expression profiles, in both rats and humans.

The commercial supplement used in the present rat study contains predominantly daidzin glucoside (about 57%). After enzymatic hydrolysis, converting the total amount of conjugated and non-conjugated soy isoflavones into non-conjugated aglycones, only daidzein and its metabolite equol were detected in the rat plasma samples. The average plasma concentrations of daidzein and equol were 0.25 and 0.38 μM, respectively (see supplementary table 7). Hosada et al. [34] and Gu et al. [35] reported that at best 3% of the total amount of soy isoflavones in the systemic circulation could be present as free bioactive aglycones. Based on this information it can be estimated that a total plasma concentration of free aglycones in rat plasma of about 19 nM (7.5 nM free diadzein aglycone and 11.4 nM free equol aglycone) could have been reached. Fig. 4 shows the estrogenic dose response curves of daidzein in vitro model systems detecting the induction of luciferase activity in different human osteosarcoma cells i.e. U2OS-ERα and U2OS-ERβ cells and proliferation in different breast cancer cells i.e. T47D-wt and T47D-ERβ cells as has been previously reported by us [36]. For the estimated total free aglycone concentration in the current rat study (plotted as vertical line in Fig. 4). It can be seen that this concentration is unlikely to induce an ERα related response, but that it could be able to induce a moderate ERβ related response. This observation might be an explanation for the low responses in gene expression, including for ERGs, as observed in the present rat study.

It should be noted, however, that this comparison of the total free aglyone concentration of 19 nM with the in vitro estrogenic response of daidzein does not consider differences in estrogenic potency between daidzein and equol. It is known that equol might have a higher estrogenic potency than daidzein, and therefore the use of the concentration of 19 nM for an expected estrogenic effect might be an underestimation. Unfortunately, no formal relative potency factor for the comparison of daidzein and equol is available. However, several authors have addressed the difference in estrogenic potency between daidzein and equol in various test systems. Hwang et al. 2006 [3] using four different cell lines (i.e. human breast cancer cell line MCF-7, human embryonic kidney cells 293, murine calvarias osteoblasts MC3T3E1, and murine

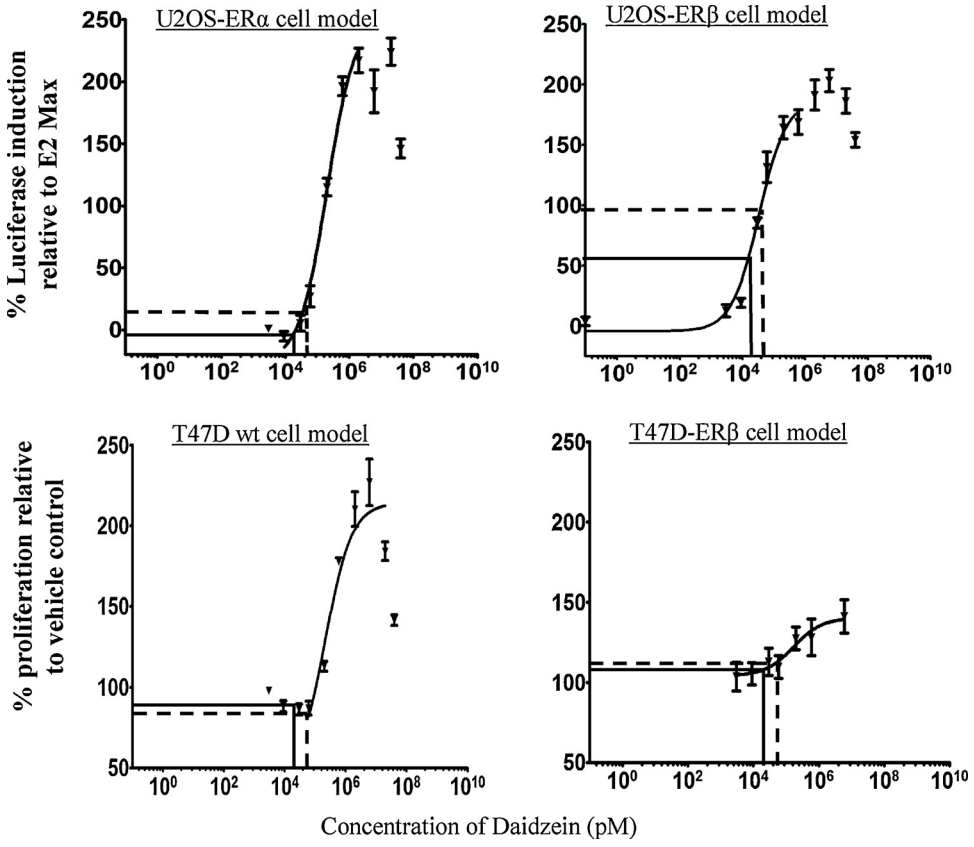

Fig. 4. Comparison of the estimated in vivo concentration of total free aglycones with the concentration-response curve of daidzein in different in vitro cell models for estrogenicity. (Figure adapted from Islam et al. [36]). The solid lines indicate the expected effect of the total free aglycones present in the rat plasma, the dotted lines taking into account the higher estrogenic potency of equol compared to daidzein.

monocytic cells RAW 264.7) transfected with the estrogen-responsive reporter gene construct 3×ERE-TATA-Luc found that the transcriptional potency of equol for the ERα receptor was 1.1 fold higher and for ERβ receptor 1.2 fold higher than that of daidzein.

Kalita and Milligan [37] using Ishikawa and yeast cells, expressed the relative potencies of different estrogenic compounds compared to estradiol (E2) and reported that the relative potency of equol is about 2–8 fold higher than that of daidzein. Comparable values, ranging from 1–4.7, have been reported in two review papers [38,39]. In contrast to these results a much higher potency factor of 100 was reported by Sathymoorthy and Wang [40] based on proliferation and mRNA expression of the oestrogen-responsive pS2 gene in MCF7 cells. Because the reported relative potencies of equol compared to daidzein, based on different test systems, differ considerably, no distinct overall potency factor can be established. But based on the available data we assumed a reasonable estimate of a factor of 10 for the relative potency of equol compared to daidzein. Thus the concentration of 19 nM as mentioned above could be converted into about 120 nM daidzein aglycone equivalents. As can be seen by the dotted lines in Fig. 4 the conclusion drawn above that the plasma levels as observed in the current rat study would not be able to induce an ERα related response, but could induce a moderate ERβ related response, is still valid.

For comparison with the rat study we also calculated the total free aglycone concentrations in plasma of human volunteers in the human intervention study where in addition to daidzein and equol, also genistein and glycitein were detected [19]. After converting the μg/mL concentrations reported by these authors to nM and using again a value of 3% for the amount of soy isoflavones that can be present in the circulation in the free form (see above), the total free aglycone concentration in these human volunteers could be estimated to be about 130 nM, on average. This is about 6.8 fold higher than the free aglycones concentration in rat plasma. Considering that the daidzein concentration in human plasma is highest (0.47 μg/mL) followed by equol (0.35 μg/mL), and that lower concentrations were found for genistein (0.18 μg) and glycitein (0.12 μg/mL), one could speculate that, even considering the higher potency of equol compared to daidzein, the concentrations of free aglycones in human plasma were also not able to induce a significant ERα related response, but that a significant ERβ related response could be possible (Fig. 4). This might explain the beneficial health effects that have been reported in several human intervention studies or randomised control trails, such as alleviation of menopausal effects [4,5,40–42]. But activation of ERβ is also associated with anti-proliferative and anti-carcinogenic effects in hormone sensitive breast cancer patients [33,43].

The observed difference in plasma concentration between rats and humans after administration of the same soy isoflavone supplement at a similar dose level (2 mg soy isoflavone/kg bw) and for the same duration (i.e. 8 weeks) can be due to species variation in ADME characteristics and/or a difference in the dosing regimen. In the present study the rats were dosed once per day in the morning and in the human intervention study the volunteers took 2 capsules in the morning and 2 in the evening after the meal. Gu et al. [35] also observed considerable species differences in plasma soy isoflavone concentrations between female adult Sprague-Dawley rats and premenopausal woman volunteers 4 h after a similar single dose; the concentration of total daidzein was 4 fold higher in human plasma compared to that of the rat. This is in line with the results of the present study. Setchell et al. [40] also found significant species differences in the circulating concentrations of aglycones between rodents and humans. Based on differences in the proportion of unconjugated soy isoflavone in plasma of humans and that of rodents, particularly in certain strains of mice, they also questioned the value of rodent models for the assessment of effects of soy isoflavone in humans. However, Setchell et al. [40] used different isoflavone sources and administration protocols to treat animals and human volunteers, and animals were treated with much higher dose levels compared to humans, leading to higher aglycone concentrations in the plasma of rats compared to humans. In addition, the adult humans span a wide range of ages (i.e. from 21 to 65 years).

We also investigated a number of pathways in the rat that might have been affected by soy isoflavone treatment. In the present study the PPAR signalling pathway was up regulated in breast uterus and liver tissues (see supplementary Table 2) indicating a positive effect of soy isoflavone treatment. However, this pathway was significantly down regulated in human PBMC following administration of the same supplement. Other studies have shown that both ERs and PPARs can influence each other and thus might lead to different effects of soy isoflavone [45,46]. It has been reported that soy isoflavone are capable to bind and activate all three isoforms namely, PPARα, PPARβ and PPARγ, of the peroxisome proliferator-activated receptors (PPARs) [44]. These PPARs are a group of transcription factors, which play an essential role in the regulation of cellular differentiation, carbohydrate, lipid and protein metabolism and tumorigenicity [44]. It was found that the p53 pathway was significantly up regulated in PBMC and breast tissue of rats (see supplementary Table 2). P-53 is an important and one of the most studied stress response pathways conserving stability of DNA, and protecting cells from DNA damage [47]. Under normal conditions this tumour suppressor gene is constitutively expressed, but it is negatively regulated by the pathway sensor called Mdm2, and then degraded. However, a variety of stress events in the cells, especially those related to DNA damage, activate a series of events that stabilize thep53 protein by inhibiting its degradation. Once activated p53 regulates divergent groups of target genes related to cell cycle arrest [48], DNA repair mechanism [49,50] and induction of apoptosis [51]. Hence activation of this p53 gene has been reported to be associated with prevention of cancer. Therefore up regulation of the p53 pathway, as observed in the present rat study, could be considered as a possible beneficial effect of exposure to soy isoflavones.

Given the fact that the correlations in gene expression in PBMC and adipose tissue between the present rat study and the human intervention study, both using the same soy isoflavones supplement, were limited, it is of importance to consider the possible limitations of the current rat study. In the rat study the animals were dosed once per day by gavage, whereas the human volunteers took the dose in two portions per day. This difference in dose regimen, together with possible effects of the circadian cycle could have influenced the gene expression. Furthermore, the comparison of the effects of soy isoflavones in rats and humans was based on the use of a similar external dose, and no consideration was given to the internal dose. As indicated above, higher plasma concentrations of soy isoflavones were found in humans compared to the rat. We previously reported that rats can metabolize soy isoflavone faster than humans [21]. So providing the same external dose on a kg/bw basis appeared to result in somewhat dissimilar plasma concentration in rats and humans, and this could have contributed to the differences in gene expression between rats and humans, as observed in the present study.

In our study we have used young ovariectomized rats and considered them as a suitable model for (post)menopausal women. Although it is a well-established model, it also have its limitations. Brinton [52] mentioned that ovariectomy is a widespread model of menopause and is typically done by removing ovaries from young reproductively competent healthy animals. The limitations he mentioned about the ovariectomy model of menopause are that in this model it is assumed that ovaries are the only organs capable of producing ovarian hormones and therefore, plasma levels of ovarian hormones are indicative of ovarian hormone concentrations in organs. However, this assumption was challenged by Caruso et al. [53], who found that changes in plasma levels of neuroactive steroid levels after gonadectomy do not necessarily reflect the steroid levels in either the peripheral or central nervous system. Secondly, in our experiments outcomes obtained from analyses of short-term ovariectomy are generalized to long-term ovarian hormone deprivation. However, this assumption ignores the adaptive responses that organs can undergo to compensate for the loss of ovarian hormones. Although removal of the ovaries in young animals is a model that broadly generalizes to human menopause. This assumption can also be challenged because adaptations can occur in the estrogen-regulated metabolic pathways during aging. In humans, ovarian hormone deprivation is chronic, in case no hormone therapy is initiated. According to the review of Brinton [52] chronic exposure in animal models is entirely feasible of human menopause. It can be thus concluded that fully developed, aged female rats may have better physiological similarities with (post)menopausal women and may therefore be a more appropriate animal model.

5. Conclusion

In the present study there are considerable differences in gene expression between various surrogate and target tissues. In addition, no cross-species early biomarkers for health effects of soy isoflavone could be identified. Based on these results it can be concluded that the use of transcriptomic data in young ovariectomized rats is challenging, but does not provide a suitable model for human risk or benefit analysis for soy isoflavone supplementation.

Conflict of interest

The authors have declared no conflict of interest.

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