Diabetologia (2006) 49: 588–597
DOI 10.1007/s00125-005-0105-3

ARTICLE

G. Bryzgalova · H. Gao · B. Ahren · J.R. Zierath · D. Galuska · T.L. Steiler · K. Dahlman-Wright · S. Nilsson · J.-Å. Gustafsson · S. Efendic · A. Khan

Evidence that oestrogen receptor-α plays an important role in the regulation of glucose homeostasis in mice: insulin sensitivity in the liver

Received: 4 March 2005 / Accepted: 7 October 2005 / Published online: 4 February 2006

Abstract *Aims/hypothesis:* We used oestrogen receptor-α (ERα) knockout (ERKO) and receptor-β (ERβ) knockout (BERKO) mice to investigate the mechanism(s) behind the effects of oestrogens on glucose homeostasis. *Methods:* Endogenous glucose production (EGP) was measured in ERKO mice using a euglycaemic–hyperinsulinaemic clamp. Insulin secretion was determined from isolated islets. In isolated muscles, glucose uptake was assayed by using radiolabelled isotopes. Genome-wide expression profiles were analysed by high-density oligonucleotide microarray assay, and the expression of the genes encoding stearoyl-CoA desaturase 1 and the Leptin receptor (*Scd1* and *Lepr*, respectively) was confirmed by RT-PCR. *Results:* ERKO mice had higher fasting blood glucose, plasma insulin levels and IGT. The plasma leptin level was increased, while the adiponectin concentration was decreased in ERKO mice. Levels of both glucose- and arginine-induced insulin secretion from isolated islets were similar in ERKO and wild-type mice. The euglycaemic–hyperinsulinaemic clamp revealed that suppression of EGP by increased insulin levels was blunted in ERKO mice, which suggests a pronounced hepatic insulin resistance. Microarray analysis revealed that in ERKO mice, the genes involved in hepatic lipid biosynthesis were upregulated, while genes involved in lipid transport were downregulated. Notably, hepatic *Lepr* expression was decreased in ERKO mice. In vitro studies showed a modest decrease in insulin-mediated glucose uptake in soleus and extensor digitorum longus (EDL) muscles of ERKO mice. BERKO mice demonstrated normal glucose tolerance and insulin release. *Conclusions/interpretation:* We conclude that oestrogens, acting via ERα, regulate glucose homeostasis mainly by modulating hepatic insulin sensitivity, which can be due to the upregulation of lipogenic genes via the suppression of *Lepr* expression.

Keywords Oestrogen receptors · Glucose homeostasis · Insulin resistance · Leptin receptor · Stearoyl-CoA desaturase 1

Abbreviations EGP: endogenous glucose production · ERα: oestrogen receptor-α · ERβ: oestrogen receptor-β · ERKO: oestrogen receptor-α knockout mice · BERKO: oestrogen receptor-β knockout mice · EDL: extensor digitorum longus muscle · GO: gene ontology · KHB: Krebs–Henseleit bicarbonate buffer

G. Bryzgalova · S. Efendic · A. Khan
Department of Molecular Medicine, Karolinska Hospital,
Karolinska Institute,
Stockholm, Sweden

H. Gao · K. Dahlman-Wright
Department of Biosciences, Karolinska Institute,
Novum, Huddinge, Sweden

B. Ahren
Department of Medicine, Lund University,
Lund, Sweden

J. Zierath · D. Galuska · T. Steiler
Department of Surgical Sciences and Department of Physiology
and Pharmacology, Karolinska Institute,
Stockholm, Sweden

S. Nilsson
KaroBio AB,
Novum, Huddinge, Sweden

J.-Å. Gustafsson
Department of Medical Nutrition, Karolinska Institute,
Novum, Huddinge, Sweden

G. Bryzgalova (✉)
Department of Molecular Medicine, L6B:01 (Endocrinology),
Karolinska Hospital,
171 76 Stockholm, Sweden
e-mail: galina.bryzgalova@molmed.ki.se
Tel.: +46-8-51773398
Fax: +46-8-51773658

Introduction

Ovariectomy increases body weight and basal glucose level, and leads to IGT in mice [1, 2]. Oestrogen replacement therapy normalises these abnormalities, which implies that oestrogens play an important role in glucose metabolism [1, 2]. Several models of oestrogen insufficiency have been used to examine the mechanism(s) of these regulatory events. In male aromatase gene knockout mice, glucose intolerance and insulin resistance developed after 12 weeks of age, and were accompanied by an increase in body weight [3]. The effects of oestrogens are mediated by two receptors: ERα and ERβ. In ERKO, but not BERKO, body mass, fat deposition and cholesterol levels were increased [4]. Both male and female ERKO mice had IGT, supporting the hypothesis that the glucose-lowering effect of oestrogens is mediated by ERα [5].

The mechanism(s) behind the IGT in ERKO mice is unknown. IGT and diabetes may develop due to decreased insulin sensitivity in the liver and/or in extra-hepatic tissues (muscle, adipose tissue) [6, 7] in combination with decreased insulin secretion from pancreatic beta cells [8, 9]. Therefore, in the present work we first determined glucose tolerance in ERKO and BERKO mice. Second, we studied insulin responsiveness to glucose in isolated islets in vitro. Third, we measured glucose turnover during an euglycaemic–hyperinsulinaemic clamp in female ERKO mice to estimate hepatic and peripheral insulin sensitivity. Fourth, we determined insulin-mediated glucose uptake in vitro in isolated soleus and EDL muscles. Since insulin resistance was largely localised in the liver, we analysed genome-wide expression profiles in female ERKO and wild-type mouse livers, using high-density oligonucleotide microarrays representing ~20,000 genes to investigate the possible molecular mechanisms underlying the observed phenotypes.

Materials and methods

Animals All animal experiments were approved by the local ethical committee. Three-month-old female and male ERKO mice, female BERKO mice and their respective controls were obtained from Taconic M&B (Ejby, Denmark). All mice used in this study were of C57BL/6J background [10, 11]. ERKO mice did not express the wild-type gene encoding ERα; however, a small splice variant of the disrupted *ERα* gene along with a residual level of ERα binding was present [12].

Insulin secretion in vitro Islets were isolated from pancreas of wild-type and female ERKO mice by collagenase digestion [13]. Groups of five islets were preincubated at 37°C for 1 h in KRB, pH 7.4, containing 3.3 mmol/l glucose. The islets were then incubated in KRB for 1 h at 37°C with 3.3, 8.3 and 16.7 mmol/l glucose, or with 3.3 mmol/l glucose and 20 mmol/l arginine. After incubation, the supernatant fractions were stored at −20°C for insulin assay. Insulin was measured by RIA [14].

Euglycaemic–hyperinsulinaemic clamp Mice were anesthetised with midazolam (0.25 mg/mouse; Dormicum, Hoffman-LaRoche, Basel, Switzerland) and a combination of fluanisone (0.5 mg/mouse) and fentanyl (0.02 mg/mouse; Hypnorm, Janssen, Beers, Belgium). Thereafter, the right jugular vein and the left carotid artery were catheterised. Thirty minutes after introduction of the catheters (t=−100 min), a bolus injection of [3-^{3}H]glucose (3 MBq; Amersham Pharmacia Biotech, Amersham, UK), was given, followed by a continuous infusion of 0.056 MBq kg^{-1} min^{-1} [3-^{3}H]glucose. This infusion was continued throughout the 190 min study period, at which point a steady state was reached. At t=0 min, a blood sample was taken for the determination of insulin and [3-^{3}H]glucose concentrations, followed by an insulin infusion at the rate of 20 mU kg^{-1} min^{-1} (Actrapid, Novo Nordisk, Denmark). Blood glucose concentrations were then determined at 5 min intervals and were maintained at a concentration of 6.5 mmol/l by infusion of a solution of 2.2 mol/l glucose at a variable rate. Blood samples were taken at 60 and 90 min for the determination of insulin, and at 90 min for the determination of [3-^{3}H]glucose. Whole-body insulin sensitivity was calculated as the 60–90 min glucose infusion rate divided by the mean of the 60–90 min insulin levels. The blood samples (100 μl) taken at 0 and 90 min were deproteinised, evaporated, and resuspended in deionised water for the determination of radioactivity and glucose levels. Basal endogenous glucose production (EGP) was calculated by dividing the rate of infusion of [3-^{3}H]glucose by the plasma glucose specific activity (i.e. dpm per min divided by dpm per mg glucose). The glucose appearance at 90 min was measured by dividing the infusion rate in dpm by the plasma glucose specific activity at this time point. EGP at this time was calculated by subtracting the glucose infusion rate from the glucose appearance rate. Finally, the glucose disposal rate was calculated as the glucose appearance rate divided by the glucose concentration.

Skeletal muscle incubation procedure and assessment of glucose transport Female ERKO and wild-type mice were anaesthetised via i.p. injection of 2.5% avertin (0.02 ml/g body weight), and EDL and soleus muscles were removed for in vitro incubation. Isolated muscles were incubated for glucose uptake essentially as described for the rat epitrochlearis muscle [15].

Glucose transport was assessed using 2-deoxyglucose as described [16]. Muscles were transferred to vials containing glucose-free Krebs–Henseleit bicarbonate buffer (KHB) containing 20 mmol/l mannitol for 10 min. Thereafter, muscles were incubated in KHB containing 1 mmol/l 2-deoxy[^{3}H]glucose (3.7 GBq/ml) (American Radiolabeled Chemicals, St Louis, MO, USA) and 19 mmol/l [^{14}C]-mannitol (2.59 GBq/ml) (Moravec Biochemicals, Brea, CA, USA) for 20 min and then immediately frozen in liquid nitrogen. The extracellular space and intracellular 2-deoxyglucose concentrations were determined as previously described [15, 16]. Glucose transport activity was expressed as nmol/l 2-deoxyglucose mg^{-1} muscle h^{-1}.

Assays for plasma leptin, adiponectin and resistin Plasma leptin levels were analysed by double-antibody RIA techniques using rabbit antimouse leptin antibodies, ^{125}I-labelled leptin and mouse leptin as standard (Linco Research St Charles, MO, USA) [17]. Plasma adiponectin was measured using a RIA kit with multispecies rabbit anti-adiponectin antiserum and ^{125}I-labelled murine adiponectin as tracer (Linco Research) [18]. Recombinant mouse adiponectin was used as standard. Free and bound radioactivity was separated by use of an anti-IgG (goat anti-guinea pig) antibody. The sensitivity of the assay was 0.8 ng/ml and the CV was <8.2% at both low and high levels. Plasma resistin levels were measured by double-antibody RIA kit (Linco Research) [19], using ^{125}I-labelled murine resistin, a mouse resistin rabbit antiserum and mouse resistin as standard. The sensitivity was 0.78 ng/ml with an intra-assay CV of 3.6%.

RNA preparation Frozen livers were homogenised and total RNA was purified using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA), followed by RNeasy kits (Qiagen, Valencia, CA, USA). RNA quality was assayed using the Agilent 2100 Bioanalyzer (Agilent, Palo Alto, CA, USA).

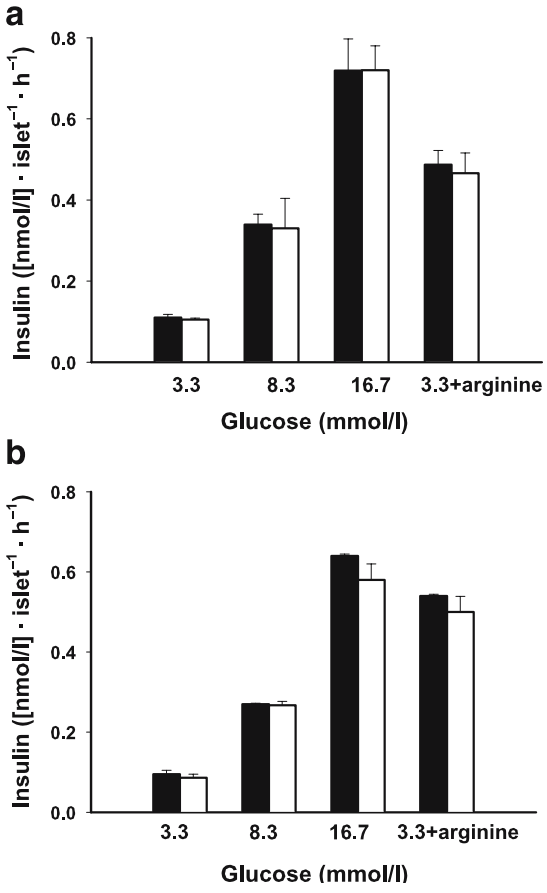

Fig. 1 Insulin secretion in isolated islets from ERKO female (*open bars*), (**a**) BERKO female (*open bars*) (**b**) and corresponding wild-type mice (*black bars*) at different glucose concentrations and in the presence of arginine. Data are presented as the means ± SEM of eight separate experiments

Table 1 Euglycaemic–hyperinsulinaemic clamp in female ERKO mice

	Wild-type	ERKO	*p* value
General information			
Number of mice analysed	7	8	
Body weight (g)	24.2±1.0	28.7±1.2	0.015
Basal level			
Basal glucose concentration (mmol/l)	10.0±1.2	14.3±0.6	0.01
Basal insulin concentration (pmol/l)	294±57	479±55	0.039
Endogenous glucose output at time *t*=0 (baseline) (μmol/l kg^{-1} min^{-1})	12.4±1.7	11.2±1.3	NS
Clamp level			
Mean glucose concentration during clamp (mmol/l)	6.0±0.1	6.4±0.3	NS
Insulin concentration during clamp (nmol/l)	11.0±2.9	10.7±2.5	NS
Weight-adjusted glucose infusion rate (nmol/l min^{-1})	15.8±3.7	3.2±1.4	0.008
Whole-body insulin sensitivity (nmol/l glucose·kg^{-1} min^{-1})/(pmol/l insulin)	16.3±2.9	2.3±1.3	0.015
Endogenous glucose output at time *t*=90 (insulin-induced) (μmol/l kg^{-1} min^{-1})	5.9±1.9	11.4±1.5	0.015
Glucose clearance rate (l kg^{-1} min^{-1})	0.017±0.002	0.014±0.002	NS

Data are presented as means ± SEM

Microarray experiment Labelled cRNA was synthesised from total RNA according to the standard Affymetrix protocol (Affymetrix, Santa Clara, CA, USA) and 15 μg of cRNA were hybridised to Mouse 430 A gene chips, washed and scanned.

Data analysis Scanned data files were analysed using MAS 5.0 software from Affymetrix. A 'coincidence call' was used to identify genes with different expression levels between ERKO and wild-type mouse livers. The coincidence call was calculated from the number of changed calls comparing all ERKO mouse samples with all wild-type samples (nine comparisons in total). Genes that were consistently changed in at least five comparisons (226 increased and 350 decreased) were selected as candidate genes predicted to be differently expressed in ERKO mouse livers compared with wild-type mouse livers. Interestingly, *Scd1* and *Lepr* genes were changed in eight and nine comparisons, respectively. The fold change reported for genes, as based on the Affymetrix analysis, is derived from the average signal log ratio for all comparisons.

The over-representation analysis approach [20] was used to test sets of related genes that might be systematically altered in ERKO livers. First, 226 increased and 350

decreased genes in ERKO vs wild-type mice were selected according to the criteria above. High-Throughput GoMiner (htgm; Zeeberg B and Qin J, unpublished results) was employed to find enrichment of changed genes involved in a particular function, using all the a priori defined gene ontology (GO) categories (http://www.geneontology.org). The original GoMiner software has been described by Zeeberg et al. [21]. Enrichment of changed genes, involved in a priori defined GO categories, is determined by a one-sided Fisher's exact test. Significantly changed GO categories with between 10 and 250 genes are selected to exclude very small and general pathways, respectively. The *p* value from the one-sided Fisher's exact test is reported, as well as the FDR test, which shows the estimated false discovery rate after multiple-comparison correction based on re-sampling technique.

Quantitative real-time RT-PCR Two micrograms of total RNA from each individual animal was reverse-transcribed into cDNA using superscript II (Invitrogen) with random hexamer primers. The expression of *Scd1* and *Lepr* was quantified using a SYBR green real-time PCR (RT-PCR) protocol with normalisation to 18S (Applied Biosystems, Foster City, CA, USA). The sequences of the primers employed to target the genes were as follows: *Scd1*: forward: TGACCTGAAAGCCGAGAAGC; reverse: ATGAAGCA CATCAGCAGGAGG; *Lepr*: forward: TGAGCAGGCG TGCCATC; reverse: GTACCCGTCAGTTTCACATGA TATATTG; 18S: forward: GCTTAATTTGACTCAACAC GGGA; reverse: AGCTATCAATCTGTCAATCCTGTCC.

Three individual animals were analysed in each group. Analysis of melting curves demonstrated amplification of one specific gene product for each primer pair.

Statistical analysis The results are expressed as means ± SEM. An unpaired Student's *t*-test was used to assess differences between wild-type and ERKO mice. All statistical tests were performed with Sigma Stat for Windows Version 1.0 (Jandel Scientific Software, Erkrath, Germany). A value of $p<0.05$ was considered significant.

Table 2 Plasma leptin, adiponectin and resistin levels in female ERKO mice

	ERKO	WT	*p* value
Leptin (ng/ml)	8.8±1.9	2.7±0.1	0.008
Adiponectin(μg/ml)	9.8±0.8	14.6±0.6	0.0004
Resistin (ng/ml)	2.2±0.1	2.75±0.22	NS

Data are presented as means ± SEM

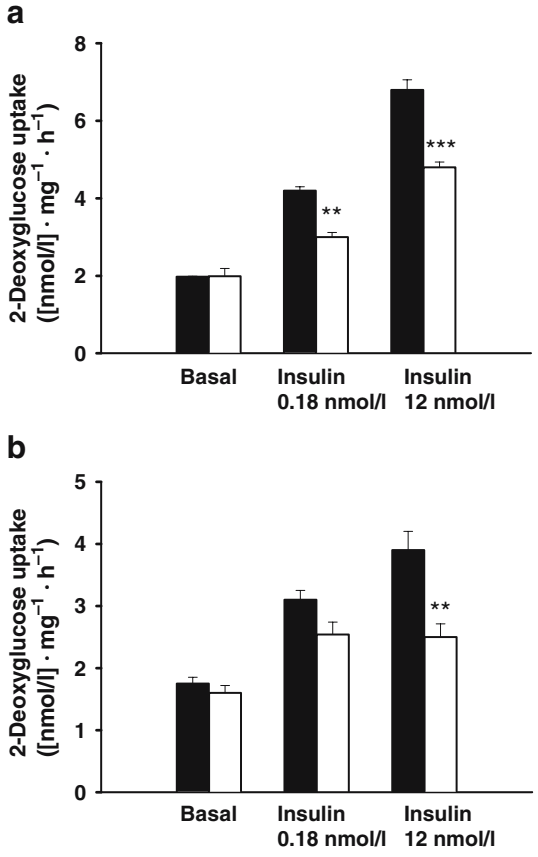

Fig. 2 Glucose uptake in skeletal muscle. **a** Soleus or **b** EDL muscles from wild-type (*black bars*) or female ERKO (*open bars*) mice were incubated for 30 min in the absence (basal) or presence of insulin (0.18 or 12 nmol/l), and 2-deoxyglucose transport was assessed as described in "Material and methods". Values represent means ± SEM for 6–10 muscles, $^{**}p<0.01$ and $^{***}p<0.001$ vs wild-type

Results

Characteristics of animals

Female ERKO mice from our colony had 20% higher body weights than wild-type mice (28.3±0.7 vs 23.8± 0.5 g, respectively, $p<0.001$). In contrast, male ERKO and wild-type mice had similar body weights (33.2±0.9 vs 33.1± 0.7 g, respectively). Female BERKO mice had normal body

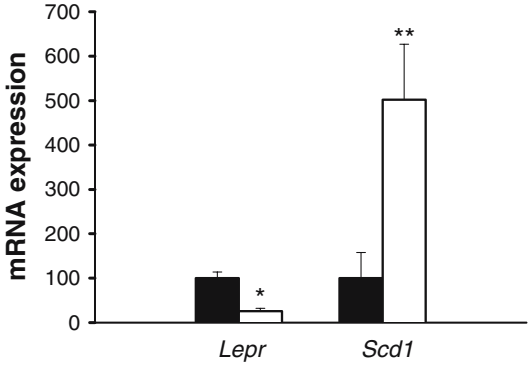

Fig. 3 RNA samples from individual mice prepared for gene profiling experiments were analysed by semi-quantitative real-time RT-PCR for *Lepr* and *Scd1* expression levels (wild-type: *black bar*; ERKO: *open bar*). Each sample was analysed in triplicate. The variation within triplicate was less than 5%. mRNA levels are expressed relative to the mean wild-type values. Data are presented as means ± SEM of three individual livers. $^{*}p<0.05$ and $^{**}p<0.01$ vs control

Table 3 Significantly changed gene ontology (GO) categories identified by High-Throughout GoMiner

GO category	*p* value	FDR	Total genes	Changed genes
Categories enriched by increased gene expression				
Steroid biosynthesis (GO:0006694)	4.62×10^{-8}	0	36	9
Lipid biosynthesis (GO:0008610)	8.72×10^{-8}	0	94	13
Lipid metabolism (GO:0006629)[a]	3.26×0^{-7}	0	250	20
Steroid metabolism (GO:0008202)[a]	9.14×10^{-7}	0	64	10
Organic acid metabolism (GO:0006082)	9.49×10^{-7}	0	197	17
Carboxylic acid metabolism (GO:0019752)	9.49×10^{-7}	0	197	17
C21-Steroid hormone biosynthesis (GO:0006700)	9.14×10^{-5}	0	12	4
Electron transport (GO:0006118)[a]	0.000107	0	177	13
Hormone metabolism (GO:0042445)	0.000109	0	23	5
C21-Steroid hormone metabolism (GO:0008207)	0.00013	0	13	4
Hormone biosynthesis (GO:0042446)	0.00013	0	13	4
Complement activation (GO:0006956)	0.000167	0	25	5
Fatty acid metabolism (GO:0006631)	0.000179	0	74	8
Alcohol metabolism (GO:0006066)	0.000218	0	118	10
Sterol biosynthesis (GO:0016126)	0.000314	0.005882	16	4
Humoral defence mechanism (sensu Vertebrata) (GO:0016064)[a]	0.000559	0.010528	32	5
Sterol metabolism (GO:0016125)[a]	0.000648	0.01	33	5
Acetyl-CoA metabolism (GO:0006084)	0.001071	0.02381	10	3
Humoral immune response (GO:0006959)	0.001591	0.045455	40	5
Coenzyme and prosthetic group metabolism (GO:0006731)[a]	0.001762	0.043478	81	7
Complement activation classical pathway (GO:0006958)	0.004542	0.065517	16	3
Amino acid derivative metabolism (GO:0006575)	0.007276	0.070588	36	4
Categories enriched by decreased gene expression				
Electron transport (GO:0006118)[a]	$1.13\text{x}10^{-7}$	0	177	22
Lipid metabolism (GO:0006629)[a]	0.000314	0	250	20
Coenzyme metabolism (GO:0006732)	0.002071	0.05	68	8
Acute-phase response (GO:0006953)	0.002789	0.044444	18	4
Regulation of cell differentiation (GO:0045595)	0.003439	0.118182	19	4
Cholesterol metabolism (GO:0008203)	0.003667	0.108333	31	5
Regulation of cell adhesion (GO:0030155)	0.003961	0.1	10	3
Humoral defence mechanism (sensu Vertebrata) (GO:0016064)[a]	0.004227	0.092857	32	5
Sterol metabolism (GO:0016125)[a]	0.004847	0.106667	33	5
Steroid metabolism (GO:0008202)[a]	0.00588	0.111765	64	7
Coenzyme and prosthetic group metabolism (GO:0006731)[a]	0.006173	0.111111	81	8
Lipid transport (GO:0006869)	0.006273	0.110526	35	5
Carboxylic acid metabolism (GO:0019752)	0.007296	0.154545	197	14
Organic acid metabolism (GO:0006082)	0.007296	0.154545	197	14
Response to pest pathogen parasite (GO:0009613)	0.008008	0.156522	159	12
Complement activation (GO:0006956)	0.009564	0.148	25	4

[a]GO categories reported to be enriched for both increased genes and decreased genes
The cut-off *p* value for significant enrichment is 0.01. FDR is the one-sided Fisher exact *p* value corrected for multiple comparisons. It is an approximation of the fraction of categories that, by random chance, would have a *p* value that was as low as that observed for the real data

weight (21.1±0.4 vs 22.1±0.4 g in controls). ERKO female and male mice had higher fasting blood glucose levels and IGT (data not shown). In contrast, fasting blood glucose and glucose tolerance were normal in BERKO mice (data not shown).

Insulin secretion in vitro In islets from wild-type and ERKO mice, glucose (3.3, 8.8, 16.7 mmol/l) stimulated insulin release in a dose-dependent manner (Fig. 1a). Insulin release was similar in wild-type and ERKO mouse islets at all glucose concentrations. At 3.3 mmol/l glucose, in the presence of arginine (20 mmol/l), insulin release was also similar between genotypes. There was no difference in insulin secretion in the presence of glucose or arginine between BERKO and wild-type mouse islets (Fig. 1b).

Euglycaemic–hyperinsulinaemic clamp Whole-body insulin sensitivity and glucose turnover were determined in female ERKO and wild-type mice using the euglycaemic

hyperinsulinaemic clamp technique (Table 1). In the basal state, plasma insulin levels were higher in ERKO than in wild-type mice, whereas EGP was similar between the groups, suggesting the presence of insulin resistance in ERKO mice. During the clamp, mean blood glucose and plasma insulin levels were similar between genotypes, whereas the glucose infusion rate needed to maintain euglycaemia was significantly lower in ERKO mice. Whole-body insulin sensitivity was markedly decreased in ERKO mice. Furthermore, suppression of EGP by the increased insulinaemia was impaired in ERKO mice. The peripheral glucose clearance rate was similar between the groups. Thus, in vivo ERKO mice display impaired whole-body insulin sensitivity, largely explained by an impaired suppression of EGP by insulin.

Glucose uptake in soleus and EDL muscles Basal glucose uptake was similar between wild-type and female ERKO mice. Insulin (0.18 and 12 nmol/l) increased 2-deoxyglucose uptake in soleus and EDL skeletal muscle in wild-type and ERKO mice (Fig. 2a,b). However, insulin-stimulated glucose uptake was suppressed in ERKO mice at both insulin concentrations (Fig. 2a).

Plasma leptin, adiponectin and resistin Circulating leptin levels were significantly higher in female ERKO mice, than wild-type mice (Table 2), whereas plasma adiponectin levels were significantly lower in ERKO mice. Resistin levels did not differ between ERKO and wild-type mice.

Differences in hepatic gene expression profiles between ERKO and wild-type mice Importantly, we identified *Scd1* and *Lepr* among a set of genes that were differently expressed. The expression level of *Scd1* was increased two-fold, while *Lepr* expression was decreased eight-fold (data not shown). The differential expression of these two genes derived from the Affymetrix platform was confirmed by real-time PCR (Fig. 3).

To get a better understanding of overall changes in gene expression, we used an over-representation analysis procedure [20] to detect coordinate changes in the expression of groups of functionally related genes or pathways employing the web-based tool 'htgm'. As shown in Table 3, 'htgm' identifies 22 GO categories enriched for increased genes in ERKO compared with wild-type mouse livers and 15 GO categories enriched for decreased genes in ERKO livers compared with wild-type mouse livers. GO categories significantly enriched for increased genes include pathways involved in steroid biosynthesis (GO:0006694), lipid biosynthesis (GO:0008610) and lipid metabolism (GO: 0006629). GO categories significantly enriched for decreased genes include pathways involved in electron transport (GO:0006118), steroid metabolism (GO:0008202) and lipid transport (GO:0006869). The enrichment of increased or decreased genes was not found in the GO categories involved in glucose metabolism, such as gluconeogenesis (GO: 0006094), glucose metabolism (GO: 0006006) and glucose transport (GO:0015758).

Table 4 Genes with changed expression in ERKO compared with wild type liver in identified gene ontology (GO) categories by High-Throughout GoMiner

Gene name	Symbol	Fold change
Genes involved in steroid biosynthesis (GO:0006694)		
Hydroxysteroid dehydrogenase-5, delta^5-3-beta	*Hsd3b5*	247.28
Hydroxysteroid dehydrogenase-2, delta^5-3-beta	*Hsd3b2*	2.1
7-Dehydrocholesterol reductase	*Dhcr7*	1.67
Hydroxysteroid dehydrogenase-6, delta^5-3-beta	*Hsd3b6*	1.65
NAD(P)-dependent steroid dehydrogenase-like	*Nsdhl*	1.64
Hydroxysteroid dehydrogenase-3, delta^5-3-beta	*Hsd3b3*	1.62
Sterol-C5-desaturase (fungal ERG3, delta-5-desaturase) homologue (*S. cerevisae*)	*Sc5d*	1.41
Hydroxysteroid (17β) dehydrogenase 12	*Hsd17b12*	1.41
Sterol-C4-methyl oxidase-like	*Sc4mol*	1.35
Genes involved in lipid biosynthesis (GO:0008610)		
Hydroxysteroid dehydrogenase-5, delta^5-3-beta	*Hsd3b5*	247.28
Elongation of very-long-chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 3	*Elovl3*	16.11
Hydroxysteroid dehydrogenase-2, delta^5-3-beta	*Hsd3b2*	2.17
Stearoyl-coenzyme A desaturase 1	*Scd1*	2.08
7-Dehydrocholesterol reductase	*Dhcr7*	1.67
Hydroxysteroid dehydrogenase-6, delta^5-3-beta	*Hsd3b6*	1.65
NAD(P)-dependent steroid dehydrogenase-like	*Nsdhl*	1.64
Hydroxysteroid dehydrogenase-3, delta^5-3-beta	*Hsd3b3*	1.62
Glycerol-3-phosphate acyltransferase, mitochondrial	*Gpam*	1.57
ATP citrate lyase	*Acly*	1.56
Sterol-C5-desaturase (fungal ERG3, delta-5-desaturase) homologue (*S. cerevisae*)	*Sc5d*	1.41
Hydroxysteroid (17β) dehydrogenase 12	*Hsd17b12*	1.41
Sterol-C4-methyl oxidase-like	*Sc4mol*	1.35
Genes involved in lipid metabolism (GO:0006629)		
Hydroxysteroid dehydrogenase-5, delta^5-3-beta	*Hsd3b5*	247.28
Elongation of very-long-chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 3	*Elovl3*	16.11
Cytochrome P450, 7b1	*Cyp7b1*	4.08
Hydroxysteroid dehydrogenase-2, delta^5-3-beta	*Hsd3b2*	2.17
Stearoyl-coenzyme A desaturase 1	*Scd1*	2.08
7-Dehydrocholesterol reductase	*Dhcr7*	1.67
Hydroxysteroid dehydrogenase-6, delta^5-3-beta	*Hsd3b6*	1.65

Table 4 (continued)

Gene name	Symbol	Fold change
NAD(P)-dependent steroid dehydrogenase-like	*Nsdhl*	1.64
Hydroxysteroid dehydrogenase-3, delta^5-3-beta	*Hsd3b3*	1.62
Glycerol-3-phosphate acyltransferase, mitochondrial	*Gpam*	1.57
ATP citrate lyase	*Acly*	1.56
Glycerol-3-phosphate dehydrogenase 1 (soluble)	*Gpd1*	1.43
Sterol-C5-desaturase (fungal ERG3, delta-5-desaturase) homologue (*S. cerevisae*)	*Sc5d*	1.41
Hydroxysteroid (17β) dehydrogenase 12	*Hsd17b12*	1.41
Sterol-C4-methyl oxidase-like	*Sc4mol*	1.35
Sterol carrier protein 2, liver	*Scp2*	1.29
Acetyl-coenzyme A acyltransferase 1	*Acaa1*	1.29
Acyl-coenzyme A oxidase 1, palmitoyl	*Acox1*	1.21
Fatty acid coenzyme A ligase, long chain 2	*Facl2*	1.19
Lipoprotein lipase	*Lpl*	1.04
Genes involved in electron transport (GO:0006118)		
Flavin-containing monooxygenase 3	*Fmo3*	−218.27
Cytochrome P450, steroid inducible 3a41	*Cyp3a41*	−45.25
Cytochrome P450, 2b9, phenobarbital inducible, type a	*Cyp2b9*	−29.65
Cytochrome P450, 3a16	*Cyp3a16*	−27.28
Cytochrome P450, 2c39	*Cyp2c39*	−12.47
Hydroxyacid oxidase (glycolate oxidase) 3	*Hao3*	−10.93
Cytochrome P450, 39a1 (oxysterol 7β-hydroxylase)	*Cyp39a1*	−3.83
Cytochrome P450, 2b20	*Cyp2b20*	−3.20
Cytochrome P450, 2b10, phenobarbital inducible, type b	*Cyp2b10*	−3.03
Cytochrome P450, 2a5	*Cyp2a5*	−2.14
P450 (cytochrome) oxidoreductase	*Por*	−1.93
Cytochrome P450, 2c38	*Cyp2c38*	−1.88
Cytochrome P450, 7a1	*Cyp7a1*	−1.66
Cytochrome P450, 2c37	*Cyp2c37*	−1.62
Flavin-containing monooxygenase 1	*Fmo1*	−1.53
Acetyl-coenzyme A dehydrogenase, long-chain	*Acadl*	−1.38
Cytochrome P450, 2g1	*Cyp2g1*	−1.38
Thioredoxin-like 2	*Txnl2*	−1.38
Signal transducer and activator of transcription 5A	*Stat5a*	−1.31
Cytochrome P450, steroid inducible 3a13	*Cyp3a13*	−1.26
Glutaredoxin 1 (thioltransferase)	*Glrx1*	−1.23
Cytochrome *b*-245, alpha polypeptide	*Cyba*	−1.11
Genes involved in steroid metabolism (GO:0008202)		
Sulfotransferase, hydroxysteroid-preferring 2	*Sth2*	−102.53
Very-low-density lipoprotein receptor	*Vldlr*	−6.59
Sterol *O*-acyltransferase 1	*Soat1*	−4.08
Cytochrome *P*450, 39a1 (oxysterol 7β-hydroxylase)	*Cyp39a1*	−3.84
Sulfotransferase family 1A, phenol-preferring, member 1	*Sult1a1*	−1.72
Cytochrome P450, 7a1	*Cyp7a1*	−1.66
3-Hydroxy-3-methylglutaryl-coenzyme A synthase 2	*Hmgcs2*	−1.40
Genes involved in lipid transport (GO:0006869)		
Very-low-density lipoprotein receptor	*Vldlr*	−6.59
Phosphatidylcholine transfer protein	*Pctp*	−1.68
Apolipoprotein A-IV	*Apoa4*	−1.54
Lipopolysaccharide-binding protein	*Lbp*	−1.49
Apolipoprotein M	*Apom*	−1.42

The fold change is derived from the average log ratio from MAS5.0

We found that among the genes involved in steroid and lipid biosynthesis, the expression of *Hsd3b5* was dramatically increased. Also, *Elovl3* expression was significantly elevated in categories of lipid biosynthesis and metabolism. The expression of *Fmo3*, *Cyp3a41* and *Cyp2b9*, which are involved in electron transport, as well as *Sth2*, which is involved in steroid metabolism, was significantly decreased.

The list of hepatic genes involved in the different categories is presented in Table 4.

Discussion

ERKO mice, irrespective of gender, developed fasting hyperinsulinaemia, hyperglycaemia and IGT. In contrast, fasting blood glucose levels and glucose tolerance were normal in BERKO mice, clearly illustrating the difference in the functions of these two oestrogen receptors in this respect. ERKO mice exhibited reduced whole-body insulin sensitivity. Insulin action on EGP was markedly impaired, while glucose uptake in muscle was slightly decreased.

Hyperglycaemia in ERKO mice can be due to impaired insulin secretion from pancreatic beta cells. Thus, in islets from ovariectomised female mice insulin secretion was significantly less in response to glucose or arginine than in control mice and this effect was abolished when the animals were treated with oestradiol and progesterone [1]. We did not find any changes in either basal or glucose- or arginine-induced insulin release in isolated ERKO mouse islets. Importantly, obese mice fed a high-fat diet maintained normoglycaemia by increasing insulin levels when compared with mice fed a low-fat diet [22]. Hence, the absence of increased glucose-stimulated insulin secretion in the presence of insulin resistance might suggest an islet dysfunction in vivo in ERKO mice.

To determine whether the insulin resistance was of hepatic and/or extra-hepatic origin, we measured glucose turnover with [3-^{3}H]glucose during basal and hyperinsulinaemic conditions. This study revealed that insulin resistance in ERKO mice could be mainly accounted for by the defective suppression of EGP. This is most likely due to insulin resistance in the liver, since other possible sources (splanchnic area, kidney) contribute only slightly to EGP [23, 24]. In agreement with this finding, oestrogens have been shown to increase hepatic insulin sensitivity by decreasing gluconeogenesis and glycogenolysis in virgin female rats and ovariectomised mice [25, 26]. Oestrogen administration decreased the expression of phosphoenolpyruvate carboxykinase, a rate-limiting enzyme in the regulation of gluconeogenesis [26]. Oestrogen treatment of *ob*/*ob* mice decreased the activity and expression of hepatic glucose-6-phosphatase, contributing to normalisation of blood glucose levels [27]. Furthermore, estrogen treatment of rats significantly decreased the hepatic activity and expression of 11β-hydroxysteroid dehydrogenase type 1, an enzyme that catalyses the conversion of inactive 11-dehydrocorticosterone to corticosterone [28].

Our clamp experiments suggest that extra-hepatic insulin resistance plays a negligible role in the development of whole-body insulin resistance in ERKO mice. However, in isolated skeletal muscle studies, insulin-mediated glucose uptake was decreased in ERKO mice, supporting the notion that ERKO mice also exhibit extrahepatic insulin resistance. Since our ERKO mice demonstrate fasting hyperglycaemia and IGT, it is possible that the insulin resistance in isolated muscle may be accounted for by glucotoxicity. In earlier studies, we have demonstrated that normalisation of blood glucose levels in diabetic GK rats restores insulin sensitivity, which we interpreted as indicating that glucotoxicity accounts for insulin resistance in the muscle [29]. Since ERKO mice show slightly increased NEFA levels [4], it is possible that lipotoxicity may also play a role in this context.

In order to study the mechanisms behind the hepatic insulin resistance in ERKO mice, we analysed genome-wide expression profiles in female ERKO and wild-type mouse livers using a high-density oligonucleotide microarray technique. This study revealed that the expression of gene categories involved in lipid biosynthesis was increased, while the expression of gene categories involved in lipid transport was decreased in ERKO mice. This is an important finding, since increased levels of lipids in the liver contribute to hepatic insulin resistance [30].

In ERKO mice, we found an increased expression of *Scd1*, an enzyme that is required for the biosynthesis of monounsaturated fatty acids. Monounsaturated fatty acids are used as substrates for the biosynthesis of triglycerides and membrane phospholipids [31]. Changes in the ratio of saturated/unsaturated fatty acids may result in alterations in membrane lipid composition and fluidity, which can modify G-protein receptor and tyrosine kinase-linked receptor signalling [32]. Recent studies suggest that in mice, increased expression of *Scd1* leads to alterations in hepatic lipid metabolism and development of the metabolic syndrome [33]. On the other hand, mice with a targeted disruption of *Scd1* gene exhibit increased insulin sensitivity and fatty acid metabolism [34]. The activity and expression of *Scd1* were highly elevated in *ob*/*ob* mice and were normalised by leptin treatment [35]. Also, the hepatic *Scd1* mRNA level was significantly increased in leptin-resistant *fa*/*fa* ZDF rats [36]. While plasma leptin levels were significantly increased in ERKO mice, we also found a decreased hepatic expression of *Lepr* in ERKO mice. Similarly, ovariectomised rats demonstrated increased plasma leptin levels, as well as a decrease in the expression of leptin receptor in the hypothalamus and adipose tissues, which was normalised by oestradiol treatment [37]. Oestradiol treatment of ovariectomised mice significantly increased hepatic *Lepr* mRNA level, even after 2 h, suggesting a direct role of estrogens on the regulation of *Lepr* (Gao H, Dahlman-Wright K, Gustaffson J-Å, unpublished results). It is therefore possible that the reciprocal expression of *Lepr* and *Scd1* may constitute an important mechanism underlying hepatic insulin resistance in ERKO mice. Ohlsson et al. [4] demonstrated slightly increased plasma NEFA levels in ERKO mice. Therefore it is possible that the increased access of NEFA to the liver may also contribute to hepatic insulin resistance.

Our microarray study revealed significant increases in hepatic expression of the *Elovl3* gene in ERKO mice. *Elov13* is necessary for the synthesis of very-long-chain fatty acids. Changes in the expression of *Elovl3* may lead to membrane defects, including the modification of phospholipid composition and signal transduction [38]. On the other hand, expression of *Sth2* (steroid metabolism pathway) was markedly decreased. This enzyme regulates the bioavailability and activity of steroid hormones, particularly, levels of hepatic oestrogens [39].

The most dramatic changes were found in the expression of *Hsd3b5* (upregulated) and *Fmo3*, *Cyp3a41* and *Cyp2b9* (downregulated). *Hsd3b5* catalyses the inactivation of steroid hormones while genes involved in electron transport such as *Fmo3* and cytochrome *P450* family play important roles in drug metabolism and detoxification in the liver. *Hsd3b5* is not expressed in control female mice [40], while *Fmo3*, *Cyp3a41* and *Cyp2b9* are only expressed in female mice [41, 42]. Therefore, the very robust changes in expression of these genes in female ERKO mice probably reflect alterations in oestrogen signalling. Importantly, the relation of the genes discussed above to hepatic glucose production is not known. In ERKO mice the expression of genes participating in regulation of gluconeogenesis was unaltered.

Mice fed a high-fat diet developed hepatic insulin resistance which was accompanied by an increase in plasma resistin levels and which was completely reversed by antisense resistin [43]. In our experiment, plasma resistin levels were normal. On the other hand, adiponectin levels were decreased in ERKO mice. It has been shown that ERKO mice had eight-fold elevated oestrogen levels compared with wild-type mice [44] and that oestrogen decreases serum adiponectin levels in mice [45]. Therefore, it is possible that in our animals, oestrogens decrease

adiponectin levels by acting through ERβ. Low levels of adiponectin are associated with obesity, insulin resistance and type-2 diabetes [46], while transgenic mice overexpressing adiponectin showed increased adiponectin levels and improved insulin sensitivity [47]. Adiponectin activates AMP-activated protein kinase resulting in phosphorylation of acetyl CoA carboxylase and thereby increased fatty acid oxidation and glucose uptake in the muscle and decreased hepatic gluconeogenesis [48]. Hence, decreased adiponectin levels may contribute to insulin resistance in the liver and/or muscle in ERKO mice.

In conclusion, our study demonstrates that the oestrogen-signalling pathway is an important regulatory factor in glucose homeostasis. Lack of ERα, but not ERβ, caused glucose intolerance and insulin resistance in both female and male mice. Thus, the ER subtypes have specialised roles in maintaining glucose homeostasis. ERKO mice display profound hepatic insulin resistance, concomitant with a moderate defect in glucose uptake in skeletal muscle. We propose that hepatic insulin resistance results from the upregulation of lipogenic genes via the suppression of *Lepr* expression.

Acknowledgements G. Bryzgalova and H. Gao contributed equally to this study. M. Reimers and B. Zeeberg are gratefully acknowledged for advice on High-Throughput GoMiner, and we would like to thank the Wallenberg Consortium North for supporting the AFFYMETRIX core facility, NOVUM. This study was supported by grants from the Swedish Research Council (K2001-70X-00034-37C and 6834), the Magnus Bergvalls Foundation, the Swedish Diabetes Association, European Union Network of Excellence CASCADE, KaroBio AB and the Swedish Cancer Fund.

References

1. Bailey CJ, Ahmed-Sorour H (1980) Role of ovarian hormones in the long-term control of glucose homeostasis: effects on insulin secretion. Diabetologia 19:475–481
2. Ahmed-Sorour H, Bailey CJ (1980) Role of ovarian hormones in the long-term control of glucose homeostasis. Interactions with insulin, glucagon and epinephrine. Horm Res 13:396–403
3. Takeda K, Toda K, Saibara T et al (2003) Progressive development of insulin resistance phenotype in male mice with complete aromatase (CYP19) deficiency. J Endocrinol 176:237–246
4. Ohlsson C, Hellberg N, Parini P et al (2000) Obesity and disturbed lipoprotein profile in estrogen receptor-α-deficient male mice. Biochem Biophys Res Commun 278:640–645
5. Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS (2000) Increased adipose tissue in male and female estrogen receptor-α knockout mice. Proc Natl Acad Sci U S A 97:12729–12734
6. Firth R, Bell P, Rizza R (1987) Insulin action in non-insulin-dependent diabetes mellitus: the relationship between hepatic and extrahepatic insulin resistance and obesity. Metabolism 36:1091–1095
7. DeFronzo RA, Bonadonna RC, Ferrannini E (1992) Pathogenesis of NIDDM. A balanced overview. Diabetes Care 15:318–368
8. Efendic S, Luft R, Wajngot A (1984) Aspects of the pathogenesis of type 2 diabetes. Endocr Rev 5:395–410
9. Mauvais-Jarvis F, Virkamaki A, Michael MD et al (2000) A model to explore the interaction between muscle insulin resistance and β-cell dysfunction in the development of type 2 diabetes. Diabetes 49:2126–2134
10. Lubahn DB, Moyer JS, Golding TS, Couse JF, Korach KS, Smithies O (1993) Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. Proc Natl Acad Sci U S A 90:11162–11166
11. Krege JH, Hodgin JB, Couse JF et al (1998) Generation and reproductive phenotypes of mice lacking estrogen receptor β. Proc Natl Acad Sci USA 95:15677–15682
12. Couse JF, Curtis SW, Washburn TF et al (1995) Analysis of transcription and estrogen insensitivity in the female mouse after targeted disruption of the estrogen receptor gene. Mol Endocrinol 9:1441–1454
13. Khan A, Hong-Lie C, Landau BR (1995) Glucose-6-phophatase activity in islets from *ob*/*ob* and lean mice and the effect of dexamethasone. Endocrinology 136:1934–1938
14. Herbert V, Lau KS, Gottlieb CW, Bleicher SJ (1965) Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab 25:1375–1384
15. Wallberg-Henriksson H, Zetan N, Henriksson J (1987) Reversibility of decreased insulin-stimulated glucose transport capacity in diabetic muscle with in vitro incubation: insulin is not required. J Biol Chem 262:7665–7671
16. Hansen P, Gulve E, Gao J, Schluter J, Mueckler M, Holloszy J (1995) Kinetics of 2-deoxyglucose transport in skeletal muscle: effects of insulin and contractions. Am J Physiol 268:C30–C35
17. Ahren B, Mansson S, Gingerich RL, Havel PJ (1997) Regulation of plasma leptin in mice: influence of age, high-fat diet, and fasting. Am J Physiol 273:R113–R120
18. Winzell MS, Nogueiras R, Dieguez C, Ahren B (2004) Dual action of adiponectin on insulin secretion in insulin-resistant mice. Biochem Biophys Res Commun 321:154–160
19. Lee JH, Bullen JW, Stoyneva VL, Mantzoros CS (2005) Circulating resistin in lean, obese, and insulin-resistant mouse models: lack of association with insulinemia and glycemia. Am J Physiol Endocrinol Metab 288:E625–E632
20. Pavlidis P, Qin J, Arango V, Mann JJ, Sibille E (2004) Using the gene ontology for microarray data mining: a comparison of methods and application to age effects in human prefrontal cortex. Neurochem Res 29:1213–1222
21. Zeeberg BR, Feng W, Wang G et al (2003) GoMiner: a resource for biological interpretation of genomic and proteomic data. Genom Biol 4:R28
22. Pierroz DD, Ziotopoulou M, Ungsunan L, Moschos S, Flier JS, Mantzoros CS (2002) Effect of acute and chronic administration of the melanocortin agonist MTII in mice with diet-induced obesity. Diabetes 51:1337–1345
23. Ahlborg G, Wahren J, Felig P (1986) Splanchnic and peripheral glucose and lactate metabolism during and after prolonged arm exercise. J Clin Invest 77:690–699
24. Gerich JE, Meyer C, Woerle HJ, Stumvoll M (2001) Renal gluconeogenesis. Its importance in human glucose homeostasis. Diabetes Care 24:382–391
25. Matute ML, Kalkhoff RK (1973) Sex steroid influence on hepatic gluconeogenesis and glycogen formation. Endocrinology 92:762–768
26. Ahmed-Sorour H, Bailey CJ (1981) Role of ovarian hormones in the long-term control of glucose homeostasis. Glycogen formation and gluconeogenesis. Ann Nutr Metab 25:208–212
27. Borthwick EB, Houston MP, Coughtrie MW, Burchell A (2001) The antihyperglycemic effect of estrone sulfate in genetically obese-diabetic (*ob*/*ob*) mice is associated with reduced hepatic glucose-6-phosphatase. Horm Metab Res 33:721–726
28. Jamieson PM, Nyirenda MJ, Walker BR, Chapman KE, Seckl JR (1999) Interections between oestradiol and glucocorticoid regulatory effects on liver-specific glucocorticoid-inducible genes: possible evidence for a role of hepatic 11β-hydroxysteroid dehydrogenase type 1. J Endocrinol 160:103–109

29. Krook A, Kawano Y, Song XM et al (1997) Improved glucose tolerance restores insulin-stimulated Akt kinase activity and glucose transport in skeletal muscle from diabetic Goto–Kakizaki rats. Diabetes 46:2110–2114
30. An J, Muoio DM, Shiota M et al (2004) Hepatic expression of malonyl-CoA decarboxylase reverses muscle, liver and whole-animal insulin resistance. Nat Med 10:268–274
31. Ntambi JM (1995) The regulation of stearoyl-CoA desaturase (SCD). Prog Lipid Res 34:139–150
32. Jump DB (2004) Fatty acid regulation of gene transcription. Crit Rev Clin Lab Sci 41:41–78
33. Biddinger SB, Almind K, Miyazaki M, Kokkotou E, Ntambi JM, Khan CR (2005) Effects of diet and genetic background on sterol regulatory element-binding protein-1c, stearoyl-CoA desaturase 1, and the development of metabolic syndrome. Diabetes 54:1314–1323
34. Ntambi JM, Miyazaki M, Stoehr JP et al (2002) Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. Proc Natl Acad Sci U S A 99:11482–11486
35. Cohen P, Miyazaki M, Socci ND et al (2002) Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. Science 297:240–243
36. Kakuma T, Lee Y, Unger RH (2002) Effect of leptin, troglitazone, and dietary fat on stearoyl CoA desaturase. Biochem Biophys Res Commun 297:1259–1263
37. Meli R, Pacilio M, Raso GM et al (2004) Estrogen and raloxifene modulate leptin and its receptor in hypothalamus and adipose tissue from ovariectomized rats. Endocrinology 145:3115–3121
38. Tvrdik P, Westerberg R, Silve S et al (2000) Role of a new mammalian gene family in the biosynthesis of very long chain fatty acids and sphingolipids. J Cell Biol 149:707–718
39. Borthwick EB, Burchell A, Coughtrie MW (1995) Differential expression of hepatic oestrogen, phenol and dehydroepiandrosterone sulphotransferases in genetically obese diabetic (*ob*/*ob*) male and female mice. J Endocrinol 144:31–37
40. Park CH, Abbaszade IC, Payne AH (1996) Expression of multiple forms of 3β-hydroxysteroid dehydrogenase in the mouse liver during fetal and postnatal development. Mol Cell Endocrinol 116:157–164
41. Falls JG, Blake BL, Cao Y, Levi PE, Hodgson E (1995) Gender differences in hepatic expression of flavin-containing monooxygenase isoforms (FMO1, FMO3, and FMO5) in mice. J Biochem Toxicol 10:171–177
42. Yamada H, Gohyama N, Honda S, Hara T, Harada N, Oguri K (2002) Estrogen-dependent regulation of the expression of hepatic Cyp2b and 3a isoforms: assessment using aromatase-deficient mice. Toxicol Appl Pharmacol 180:1–10
43. Muse ED, Obici S, Bhanot S et al (2004) Role of resistin in diet-induced hepatic insulin resistance. J Clin Invest 114:232–239
44. Couse JF, Yates MM, Walker VR, Korach KS (2003) Characterization of the hypothalamic–pituitary–gonadal axis in estrogen receptor (ER) Null mice reveals hypergonadism and endocrine sex reversal in females lacking ERalpha but not ERbeta. Mol Endocrinol 17:1039–1053
45. Combs TP, Berg AH, Rajala MW et al (2003) Sexual differentiation, pregnancy, calorie restriction, and aging affect the adipocyte-specific secretory protein adiponectin. Diabetes 52(2):268–276 Feb
46. Haluzik M, Parizkova J, Haluzik MM (2004) Adiponectin and its role in the obesity-induced insulin resistance and related complications. Physiol Res 53:123–129
47. Combs TP, Pajvani UB, Berg AH et al (2004) A transgenic mouse with a deletion in the collagenous domain of adiponectin displays elevated circulating adiponectin and improved insulin sensitivity. Endocrinology 145:367–383
48. Yamauchi T, Kamon J, Minokoshi Y et al (2002) Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 8:1288–1295