

Selective Liver Estrogen Receptor Modulation Prevents Steatosis, Diabetes, and Obesity Through the Anorectic Growth Differentiation Factor 15 Hepatokine in Mice

Maeva Guillaume, Elodie Riant, Aurelie Fabre, Isabelle Raymond-Letron, Melissa Buscato, Morgane Davezac, Blandine Tramunt, Alexandra Montagner, Sarra Smati, Rana Zahreddine, et al.

▶ **To cite this version:**

Maeva Guillaume, Elodie Riant, Aurelie Fabre, Isabelle Raymond-Letron, Melissa Buscato, et al.. Selective Liver Estrogen Receptor Modulation Prevents Steatosis, Diabetes, and Obesity Through the Anorectic Growth Differentiation Factor 15 Hepatokine in Mice. Hepatology Communications, 2019, 3 (7), pp.908-924. 10.1002/hep4.1363 . hal-02277993

HAL Id: hal-02277993

https://univ-rennes.hal.science/hal-02277993

Submitted on 4 Sep 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Selective Liver Estrogen Receptor α Modulation Prevents Steatosis, Diabetes, and Obesity Through the Anorectic Growth Differentiation Factor 15 Hepatokine in Mice

Maeva Guillaume,[1,2] Elodie Riant,[1] Aurélie Fabre,[1] Isabelle Raymond-Letron,[3] Melissa Buscato,[1] Morgane Davezac,[1] Blandine Tramunt,[1] Alexandra Montagner,[1] Sarra Smati,[1,4] Rana Zahreddine,[1] Gaëlle Palierne,[5] Marie-Cécile Valera,[1] Hervé Guillou,[4] Françoise Lenfant,[1] Klaus Unsicker,[6] Raphaël Metivier,[5] Coralie Fontaine,[1] Jean-François Arnal,[1] and Pierre Gourdy[1,7]

Hepatocyte estrogen receptor α (ERα) was recently recognized as a relevant molecular target for nonalcoholic fatty liver disease (NAFLD) prevention. The present study defined to what extent hepatocyte ERα could be involved in preserving metabolic homeostasis in response to a full (17β-estradiol [E2]) or selective (selective estrogen receptor modulator [SERM]) activation. Ovariectomized mice harboring a hepatocyte-specific *ERα* deletion (*LERKO* mice) and their wild-type (WT) littermates were fed a high-fat diet (HFD) and concomitantly treated with E2, tamoxifen (TAM; the most used SERM), or vehicle. As expected, both E2 and TAM prevented all HFD-induced metabolic disorders in WT mice, and their protective effects against steatosis were abolished in *LERKO* mice. However, while E2 still prevented obesity and glucose intolerance in *LERKO* mice, hepatocyte *ERα* deletion also abrogated TAM-mediated control of food intake as well as its beneficial actions on adiposity, insulin sensitivity, and glucose homeostasis, suggesting a whole-body protective role for liver-derived circulating factors. Moreover, unlike E2, TAM induced a rise in plasma concentration of the anorectic hepatokine growth differentiation factor 15 (Gdf15) through a transcriptional mechanism dependent on hepatocyte ERα activation. Accordingly, ERα was associated with specific binding sites in the *Gdf15* regulatory region in hepatocytes from TAM-treated mice but not under E2 treatment due to specific epigenetic modifications. Finally, all the protective effects of TAM were abolished in HFD-fed *GDF15*-knockout mice. *Conclusion:* We identified the selective modulation of hepatocyte ERα as a pharmacologic strategy to induce sufficient anorectic hepatokine Gdf15 to prevent experimental obesity, type 2 diabetes, and NAFLD. (*Hepatology Communications* 2019;3:908-924).

As a key regulator of energy and glucose homeostasis, estrogen receptor α (ERα) is now considered a relevant target to develop new therapeutic approaches for obesity-related metabolic disorders, such as type 2 diabetes and nonalcoholic fatty liver disease (NAFLD).[1] Understanding the mechanisms involved in ERα-mediated metabolic protection thus remains a crucial challenge to

Abbreviations: AF, activation function; Angptl, angiopoietin-like; BS, binding site; CBP, cyclic adenosine monophosphate response element-binding protein; ChIP, chromatin immunoprecipitation; CoA, coenzyme A; Cyp17a1, cytochrome P450, family 17, subfamily a, polypeptide 1; Dgat2, diacylglycerol O-acyltransferase 2; E2, 17β-estradiol; Enho, energy homeostasis associated; ERα, estrogen receptor α; Fgf21, fibroblast growth factor 21; Gck, glucokinase; Gdf15, growth differentiation factor 15; GDF15KO, growth differentiation factor 15-deficient; H&E, hematoxylin and eosin; H3K27ac, twenty-seventh amino acid in histone H3, acetylated; HDL, high-density lipoprotein; HFD, high-fat diet; Igf, insulin-like growth factor; KO, knockout; LDL, low-density lipoprotein; LERKO, mice with hepatocyte-restricted ERα deletion; mRNA, messenger RNA; NAFLD, nonalcoholic fatty liver disease; Nr1h, nuclear receptor subfamily 1, group H; Pltp, phospholipid transfer protein; Ppar, peroxisome proliferator activator receptor; SERM, selective estrogen receptor modulator; Srebf1, sterol regulatory element binding transcription factor 1; TAM, tamoxifen; VEH, vehicle; WT, wild type.

Received February 15, 2019; accepted March 28, 2019.
Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1363/suppinfo.

optimize pharmacologic strategies for ERα selective modulation.[2]

Interestingly, recent studies highlighted the critical role of hepatic ERα signaling in the protective effects conferred by estrogens against high-fat diet (HFD)-induced steatosis and insulin resistance. Indeed, silencing ERα expression in the liver using adenoviral short hairpin RNA has been shown to markedly increase hepatic triglyceride accumulation in HFD-fed C57BL/6 female mice, while overexpression of human ERα reduced the level of steatosis in obese ob/ob female mice.[3] Zhu et al.[4] then reported that both female and male mice with hepatocyte-restricted *ERα* deletion (*LERKO*) are prone to steatosis and insulin resistance in response to HFD feeding. Noteworthy, 17β-estradiol (E2) administration failed to preserve ovariectomized *LERKO* female mice from fatty liver and insulin resistance, although this treatment still exerted a significant protection against adipose tissue accumulation and glucose intolerance.[4]

As a member of the nuclear receptor superfamily, ERα acts as a transcription factor that regulates gene transcription through two activation functions (AFs), namely ERα-AF1 and ERα-AF2.[5] ERα-AF1 and ERα-AF2 are both fully activated by E2, but their respective roles in gene expression regulation depend on ligands and cell types. Using mouse models with a selective ERα-AF1 or ERα-AF2 deletion, we demonstrated that the prevention of HFD-induced obesity and insulin resistance by E2 required ERα-AF2 activation, whereas ERα-AF1 appeared dispensable.[6] Besides natural estrogens, several drugs, known as selective estrogen receptor modulators (SERMs), acting as agonists or antagonists in a tissue-specific manner have been developed for clinical use. Nevertheless, their abilities to activate or repress ERα-AF1 or ERα-AF2 remain largely unknown, except for tamoxifen (TAM).[5] Indeed, ERα-AF2 is the direct target of TAM antagonism, whereas its agonistic activity, which depends on both cell types and target genes, results from ERα-AF1 activation.[7]

Supported by INSERM (J.F.A & P.G.), Université de Toulouse and Faculté de Médecine de Toulouse (J.F.A & P.G.), Fondation de France (C.F.), Conseil Régional Midi-Pyrénées/Occitanie (P.G.), Fondation pour la Recherche Médicale (J.F.A.), Société Francophone du Diabète (A.M. & P.G.), and Agence Nationale de la Recherche (C.F.). The nuclear magnetic resonance facility is part of the Genotoul-Ibisa PICT (Integrated Screening Platform of Toulouse) platform and was funded by the Centre National de la Recherche Scientifique, Région Midi-Pyrénées, and European structural funds.

© 2019 The Authors. Hepatology Communications published by Wiley Periodicals, Inc., on behalf of the American Association for the Study of Liver Diseases. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep4.1363

Potential conflict of interest: Nothing to report.

ARTICLE INFORMATION:

From the [1]Institut des Maladies Métaboliques et Cardiovasculaires, Unité Médicale de Recherche 1048, Institut National de la Santé et de le Recherche Médicale (INSERM), Université de Toulouse III, Toulouse, France; [2]Service d'Hépato-gastro-entérologie, Centre Hospitalier Universitaire de Toulouse, Toulouse, France; [3]STROMALab, Centre National de la Recherche Scientifique ERL5311, Etablissement Français du Sang, Ecole Nationale Vétérinaire de Toulouse, Institut National de la Santé et de le Recherche Médicale (INSERM) U1031, Université de Toulouse III, Toulouse, France; [4]Institut National de La Recherche Agronomique, Unité Médicale de Recherche 1331, ToxAlim, Université de Toulouse, Toulouse, France; [5]Equipe SP@RTE, Unité Médicale de Recherche 6290, Institut de Genétique et Développement de Rennes, Université de Rennes 1, Rennes, France; [6]Institute of Anatomy and Cell Biology, Department of Molecular Embryology, University of Freiburg, Freiburg, Germany; [7]Service de Diabétologie, Maladies Métaboliques et Nutrition, Centre Hospitalier Universitaire de Toulouse, Toulouse, France.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Pierre Gourdy, M.D., Ph.D.
Institut des Maladies Métaboliques et Cardiovasculaires
Unité Médicale de Recherche 1048, Team 9
Institut National de la Santé et de le Recherche Médicale (INSERM)
1 avenue Jean Poulhès, BP 84225
31432 Toulouse Cedex 4, France
E-mail: pierre.gourdy@inserm.fr
Tel.: +33 561 323 740

We recently showed that chronic TAM administration prevents HFD-induced obesity, steatosis, insulin resistance, and glucose intolerance in ovariectomized female C57BL/6 mice, along with a significant reduction in food intake.[8] All the protective effects of TAM were abrogated in *ERα*$^{-/-}$ and *ERα-AF1*$^{-/-}$ mice,[8] thus attesting that TAM-induced metabolic protection is specifically mediated by ERα-AF1. However, the specific contribution of hepatocyte ERα to the protection conferred by SERMs, such as TAM, against HFD-induced metabolic disorders still remain to be investigated.

The present study aimed at defining to what extent hepatocyte ERα could be involved in the prevention of steatosis and in the preservation of whole-body metabolic homeostasis in response to a selective (SERM) mode of activation compared to a full (E2) activation. To this end, we combined the use of pharmacologic tools (E2 and TAM) and transgenic mouse models to demonstrate that, in contrast to the results obtained with E2, hepatocyte ERα not only mediates the protective effect of TAM against steatosis but is also absolutely and unexpectedly required to prevent HFD-induced obesity and glucose intolerance. Noteworthy, this overall metabolic protective action conferred by the selective modulation of hepatocyte ERα requires the anorectic hepatokine growth differentiation factor 15 (Gdf15), secretion of which is sharply enhanced by TAM.

Materials and Methods

ANIMALS, TREATMENT, AND DIET

Female mice with a hepatocyte-specific deletion of ERα (*LERKO*, n = 40)[9] and *Gdf15*-deficient mice (*GDF15KO*, n = 23)[10] (see Supporting Methods) as well as their respective wild-type (WT) littermates (*LER*$^{+/+}$, n = 40; *GDF15*$^{+/+}$, n = 14) underwent bilateral ovariectomy at 4 weeks of age to standardize the exposure to ERα ligands. All mice were then subcutaneously implanted with pellets releasing either TAM (1.2 mg/kg/day), E2 (80 μg/kg/day), or vehicle (VEH) for either 6 (*GDF15KO*) or 12 weeks (*LERKO)* and concomitantly fed an HFD (energy content: 45% fat, 20% protein, and 35% carbohydrate; 3.7 kcal/g; Research Diets, New Brunswick, NJ).

IN VIVO EXPERIMENTAL PROCEDURES

Mice were anesthetized with an intraperitoneal injection of ketamine (10 mg/kg; Merial, Lyon, France) and xylazine (1 mg/kg; Sigma-Aldrich, Isle d'Abeau Chesnes, France) for ovariectomy and with exposition to 2% isoflurane for pellet implantation. Food intake and body weight were recorded weekly. One week before being killed, body composition (whole body fat and lean masses) was analyzed by EchoMRI in live animals. Intraperitoneal glucose tolerance tests were performed on the following day in overnight-fasted mice. Blood glucose concentrations were monitored with a glucose meter (Roche Diagnostic, Meylan, France) –30, 0, 30, 60, and 90 minutes after glucose injection (1 g/kg body weight). In some experiments, basal metabolism was evaluated by indirect calorimetry 1 week before being killed and after 24 hours of acclimatization in individual cages. Oxygen consumption (VO_2), carbon dioxide production (VCO_2), and food and water intake were measured (Phenomaster; TSE-Systems) in individual mice at 10-minute intervals during a 24-hour period at constant temperature (20°C). We calculated the respiratory exchange ratio as VCO_2 /VO_2 and energy expenditure (kilocalories of heat produced). Ambulatory physical activity was monitored by an infrared photocell beam interruption method. All mice were killed at 11:00 AM, after 3 hours of fasting with free access to water. Blood samples were collected from the retro-orbital venous plexus and stored at –20°C. Mice were euthanized by cervical dislocation, and organs were carefully removed, weighed, frozen in liquid nitrogen, and stored at –80°C.

LIVER TISSUE HISTOLOGY

Liver tissues were quickly excised, fixed in 10% buffered formalin, and embedded in paraffin, and 3-μm sections were stained with hematoxylin and eosin (H&E). Additional fresh liver samples were immersed in Tissue-Tek optimal cutting temperature compound (Sakura, Japan) and then frozen in isopentane cooled by liquid nitrogen; cryosections (7 μm) were then stained with Oil Red O to assess neutral lipid accumulation, as described with minor modifications.[11] Images of each sample were obtained at original magnifications of ×150 and ×400 with an Eclipse Ci Nikon microscope and using a DS-FI camera driven by NIS-AR element

software (Nikon) (H&E sections) or scanned (Oil Red O sections) with a nanozoomer scanner (Hamamatsu Photonics, Hamamatsu, Japan) and analyzed with NDP view software (Hamamatsu Photonics).

LIVER LIPID CONTENT ANALYSIS

Hepatic levels of triglycerides, free cholesterol, and cholesterol esters were determined using Bligh and Dyer methodology,[12] and lipid extracts were analyzed by gas–liquid chromatography as described.[13]

BIOCHEMICAL ANALYSES

Plasma samples were used to measure alanine aminotransferase and lipid profiles (triglycerides, total cholesterol, and high-density lipoprotein (HDL) cholesterol). Plasma insulin and adipokines (resistin, leptin, adiponectin) levels were determined using the Multiplex Immunoassay Technology Xmap (MADKMAG-71K-05 and MADPNMAG-70K-01, MILLIPEX; Millipore, Saint-Quentin-en-Yveline, France). Serum Gdf15 levels were determined with a commercial enzyme-linked immunosorbent assay kit (MGD150; R&D Systems Europe, Abingdon, United Kingdom) according to the manufacturer's instructions.

OTHER METHODS

Methods used for ERα protein determination by western blot on isolated hepatocytes, gene expression analysis, and chromatin immunoprecipitation (ChIP) assays are detailed in Supporting Methods.

STATISTICAL ANALYSIS

Results are expressed as mean ± SEM. Statistical analyses were performed using GraphPad Prism, version 5.00, for Windows (GraphPad Software, San Diego, CA; www.graphpad.com). A Student *t* test was used to compare the respective effects of TAM and E2 with the effect of VEH within each genotype (WT or knockout [KO] mice). To test the interaction between treatments and genotypes for body weight evolution and glycemic changes during intraperitoneal glucose tolerance tests, a two-way analysis of variance with repeated measures was carried out. In the case of interaction between treatment and genotype, Bonferroni post-hoc tests were subsequently performed. $P < 0.05$ was considered statistically significant.

Results

BOTH E2 AND TAM PREVENT HFD-INDUCED STEATOSIS THROUGH HEPATOCYTE ERα ACTIVATION

LERKO mice were first used to determine whether ERα signaling in hepatocytes contributes to the prevention of HFD-induced steatosis conferred by TAM and E2. Demonstrating the selective *ERα* deletion in this mouse model, ERα messenger RNA (mRNA) levels were decreased by 80% in total livers and primary hepatocytes from *LERKO* mice compared to their WT littermates, while no differences were observed in other tissues (Supporting Fig. S1A). Furthermore, ERα protein expression was not detected in the culture of primary hepatocytes from *LERKO* mice (Supporting Fig. S1B). Both E2 and TAM protected ovariectomized WT mice from HFD-induced steatosis, as illustrated by histologic staining (Fig. 1A) and confirmed by a significant decrease in intrahepatic concentrations of triglycerides (Fig. 1B). Prevention of steatosis development by either TAM or E2 was abolished in *LERKO* mice (Fig. 1A,B). Histologic analyses found no lobular inflammation, hepatocyte ballooning, or fibrosis, irrespective of the treatment and genotype (data not shown). Accordingly, no significant changes were observed in the hepatic expression of a set of genes involved in inflammation and fibrosis (Supporting Fig. S2; data not shown). Finally, in TAM-treated mice, we checked that hepatocyte *ERα* deletion does not alter the expression of genes encoding P450 enzymes that contribute to metabolize this SERM in the liver (Supporting Fig. S3). These data first confirm that hepatocyte ERα plays an important role in the hepatoprotective actions of E2[4] and then demonstrate that TAM-induced prevention of steatosis[8] also results from ERα activation in hepatocytes.

INVOLVEMENT OF HEPATOCYTE ERα IN THE REGULATION OF HEPATIC METABOLIC PATHWAYS BY TAM AND E2

To gain further insights into the contribution of hepatocyte ERα to the protective effects of E2 and TAM against HFD-induced steatosis, we explored the regulation of the main hepatic metabolic pathways,

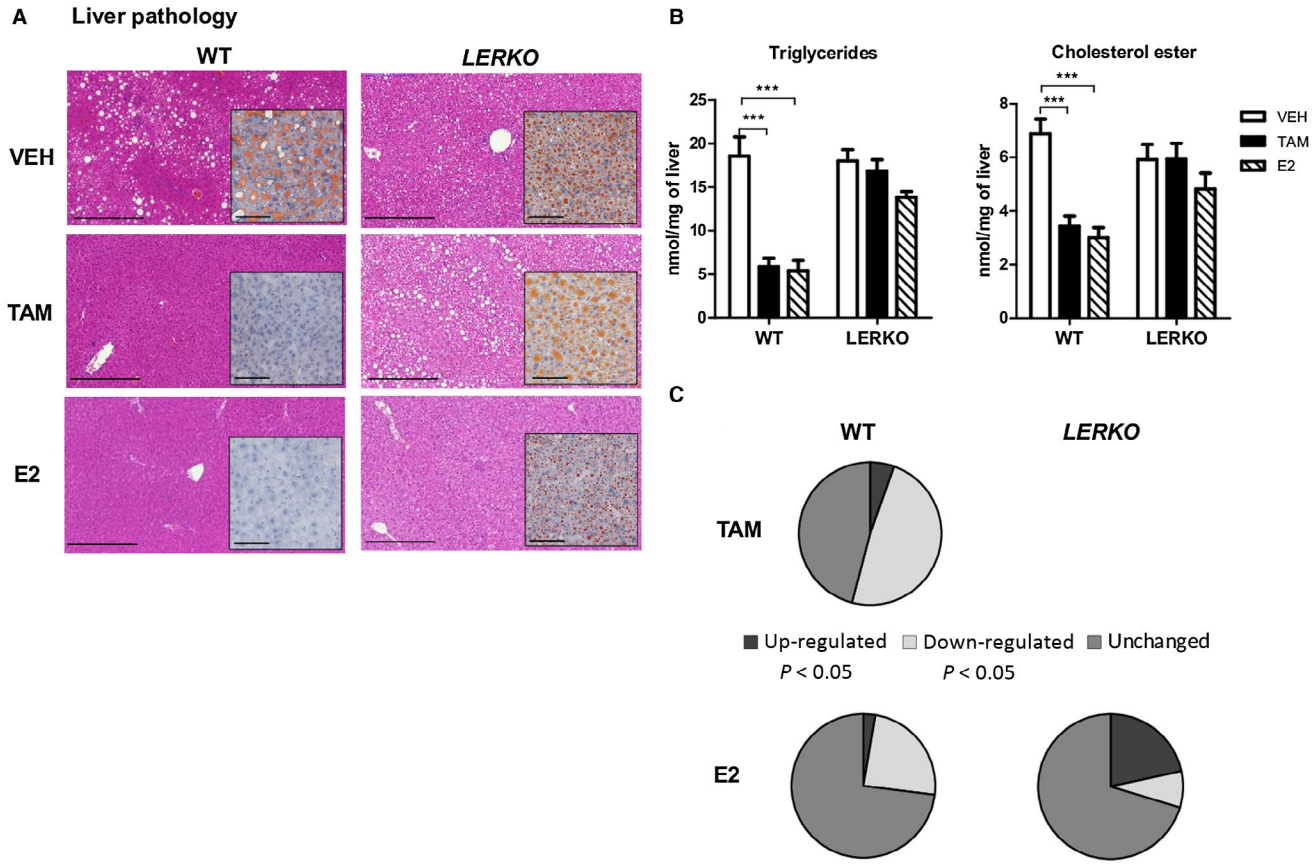


FIG. 1. Prevention of HFD-induced steatosis by either E2 or TAM requires hepatocyte ERα activation. *LERKO* mice (n = 40) and their respective WT littermates (n = 40) were fed an HFD and concomitantly treated with TAM, E2, or VEH for 12 weeks. (A) Liver histology: representative images of H&E (scale bars, 100 μm) and Oil Red O staining (insets; scale bars, 50 μm). (B) Intrahepatic lipid content. (C) Liver mRNA expression (37 genes involved in metabolic pathways). Genes were defined as up- or down-regulated if the fold change compared to control was statistically significant ($P < 0.05$, Student *t* test). Data are expressed as mean ± SEM. * indicates differences between VEH and treated (TAM or E2) mice within each genotype. ***$P < 0.001$.

focusing on a set of 37 selected genes involved in lipid and glucose metabolism. In WT-treated mice, 20/37 and 10/37 genes were significantly regulated by TAM and E2, respectively (summarized in Fig. 1C). In our experimental settings, no significant changes were observed in the mRNA expression levels of acetyl-coenzyme A (CoA) acyltransferase 2 (*Acaa2*), acyl-CoA dehydrogenase, long-chain (*Acadl*), apolipoprotein B (*Apob*), carnitine palmitoyltransferase 1A (*Cpt1a*), cytochrome P450, family 17, subfamily a, polypeptide 1 (*Cyp17a1*), fatty acid binding protein 4, adipocyte (*Fabp4*), glucose-6-phosphatase, catalytic (*G6pc*), 3-hydroxy-3-methylglutaryl-CoA reductase (*Hmgcr*), insulin receptor (*Insr*), insulin receptor substrate 2 (*Irs2*), lipoprotein lipase (*Lpl*), MLX interacting protein-like (*Mlxipl*), nuclear receptor subfamily 1, group H, member 4 (*Nr1h4*), patatin-like phospholipase domain containing 2 (*Pnpla2*), sirtuin 1 (*Sirt1*), and solute carrier family 2 member 4 (*Slc2a4*) following either TAM or E2 administration (data not shown). In TAM-treated WT mice, genes involved in *de novo* lipogenesis and lipid synthesis (acetyl-CoA carboxylase alpha [*Acaca*], CCAAT/enhancer binding protein, alpha [*Cebpa*], diacylglycerol O-acyltransferase 2 [*Dgat2*], fatty acid synthase [*Fasn*], *Nr1h3,* phosphatidylethanolamine N-methyltransferase [*Pemt*], peroxisome proliferator activator receptor delta [*Ppard*], stearoyl-CoA desaturase 1 [*Scd1*], and sterol regulatory element binding transcription factor 1 [*Srebf1*]), cholesterol metabolism and lipoprotein assembly (*Apoa1*, *Apoa4*, *Apoa5,* and phospholipid transfer protein [*Pltp*]), lipid transport (*Fabp1*) and catabolism (*Acacb*,

acyl-CoA oxidase 1, palmitoyl (*Acox1*), peroxisome proliferator activated receptor alpha [*Ppara*]), and glucose metabolism (glucokinase [*Gck*]) were down-regulated compared to VEH-treated mice (Supporting Fig. S4). Inversely, genes encoding for gluconeogenic enzyme (phosphoenolpyruvate carboxykinase 1, cytosolic [*Pck1*]*)* and hepatic insulin sensitivity (leptin receptor [*Lepr*]) were up-regulated by TAM. Noteworthy, TAM-sensitive genes were all dependent on hepatocyte ERα because their regulations were abolished in *LERKO* mice (Fig. 1C; Supporting Fig. S4). In E2-treated WT mice, some genes involved in *de novo* lipogenesis and lipid synthesis (*Acaca*, *Dgat2*, *Fasn*, *Scd1,* and *Srebf1*), cholesterol metabolism and lipoprotein assembly (*Pltp*), lipid catabolism (*Acacb*, *Ppara*), and glucose metabolism (*Gck*) were down-regulated, while *Cyp17a1,* a key gene for steroidogenesis, was up-regulated compared to VEH-treated mice (Supporting Fig. S4). The up-regulation of *Cyp17a1* and the down-regulation of *Acaca, Acacb, Dgat2, Srebf1, Pltp, Ppara,* and *Gck* by E2 were abrogated in *LERKO* mice, indicating that only 80% of E2-regulated genes were dependent on hepatocyte ERα activation (Fig. 1C; Supporting Fig. S4). Based on the protection conferred by TAM and E2 against HFD-induced steatosis, both molecules thus down-regulate the expression of key lipogenic genes. Although other cellular targets could contribute to E2-mediated control of these hepatic metabolic pathways, the present data demonstrate that their regulation by TAM entirely relies on the direct activation of hepatocyte ERα.

HEPATOCYTE ERα MEDIATES PROTECTIVE EFFECTS OF TAM ON WHOLE-BODY ENERGY AND GLUCOSE HOMEOSTASIS

We next considered the role of hepatocyte ERα in the preventive actions of TAM and E2 against HFD-induced systemic disorders, namely obesity, insulin resistance, and hyperglycemia. In agreement with other reports,[6,8] both molecules protected ovariectomized WT female mice from body weight gain (Fig. 2A), but only TAM administration significantly decreased food intake (Fig. 2B). E2 and TAM reduced fat mass accumulation to a similar extent (Fig. 2C), including subcutaneous and visceral sites (Fig. 2D), without any impact on lean mass (Fig. 2C). As expected, the two treatments also prevented HFD-induced glucose intolerance and insulin resistance in WT mice (Fig. 2E). E2 administration still protected *LERKO* female mice from HFD-induced obesity (Fig. 2A,C,D), as reported,[4] and from glucose intolerance, although to a lesser extent than in WT mice (Fig. 2E). In contrast, the preventive effects of TAM on body weight gain (Fig. 2A), fat mass accumulation (Fig. 2C,D), insulin resistance, and glucose intolerance (Fig. 2E) as well as its anorectic effect (Fig. 2B) were totally abrogated in *LERKO* mice. Plasma lipid profiles in WT mice revealed that TAM significantly reduced total and HDL-cholesterol levels together with an increase in low-density lipoprotein (LDL)-cholesterol concentrations, while E2 treatment was associated with a decrease in HDL-cholesterol and an increase in LDL-cholesterol levels (Table 1). Adiponectin and leptin plasma concentrations were significantly decreased by both treatments in WT mice, while resistin levels were only reduced by TAM administration (Table 1). In *LERKO* mice, all the effects of E2 on plasma lipid and adipokine profiles were preserved, whereas those of TAM were abolished except for adiponectin concentration that was still reduced (Table 1). These results indicate that specific activation of hepatocyte ERα is dispensable for E2-mediated prevention of obesity and associated metabolic disorders but is absolutely required for the systemic effects of TAM, including the control of food intake as well as the preservation of whole-body energy and glucose homeostasis.

HEPATOCYTE ERα IS REQUIRED FOR THE EFFECT OF TAM ON FOOD INTAKE AND ENERGY EXPENDITURE

To further characterize the involvement of hepatocyte ERα in the respective influence of TAM and E2 on energy homeostasis, HFD-fed WT and *LERKO* mice were assessed in metabolic cages, including indirect calorimetry measurements. No significant change in respiratory exchange ratio was observed with either TAM or E2 (Fig. 3A). E2 did not influence energy expenditure (Fig. 3B) but tended to increase nocturnal physical activity, both in WT and in *LERKO* mice (Fig. 3C), suggesting that metabolic effects induced by E2 involve central mechanisms independent of hepatocyte ERα activation. Conversely, TAM tended to decrease nocturnal

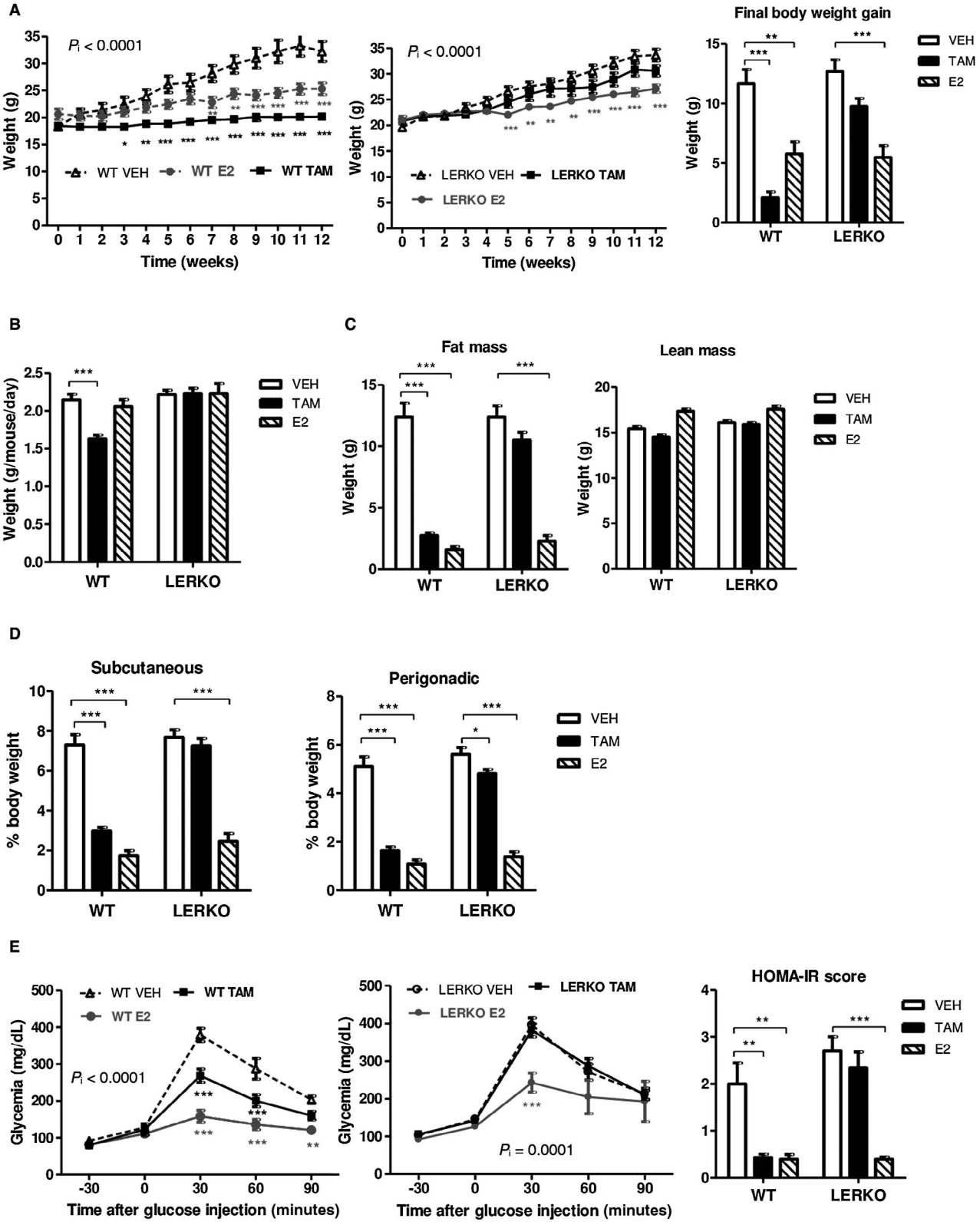

A
Pi < 0.0001
Weight (g)
Time (weeks)
WT VEH
WT E2
WT TAM
Pi < 0.0001
Weight (g)
Time (weeks)
LERKO VEH
LERKO TAM
LERKO E2
Final body weight gain
Weight (g)
VEH
TAM
E2
WT
LERKO
B
Weight (g/mouse/day)
VEH
TAM
E2
WT
LERKO
C
Fat mass
Weight (g)
WT
LERKO
Lean mass
Weight (g)
VEH
TAM
E2
WT
LERKO
D
Subcutaneous
% body weight
WT
LERKO
Perigonadic
% body weight
VEH
TAM
E2
WT
LERKO
E
Glycemia (mg/dL)
WT VEH
WT TAM
WT E2
Pi < 0.0001
Time after glucose injection (minutes)
Glycemia (mg/dL)
LERKO VEH
LERKO TAM
LERKO E2
Pi = 0.0001
Time after glucose injection (minutes)
HOMA-IR score
VEH
TAM
E2
WT
LERKO

FIG. 2. Hepatocyte ERα mediates the protective effects of TAM but not of E2 against HFD-induced obesity, insulin resistance, and glucose intolerance. *LERKO* mice (n = 40) and their respective WT littermates (n = 40) were fed an HFD and treated with TAM, E2, or VEH for 12 weeks. (A) Body weight evolution and final body weight gain; (B) mean food intake; (C) body composition; (D) adipose tissue distribution; (E) intraperitoneal glucose tolerance tests and HOMA-IR score. Data are expressed as mean ± SEM. $P_i < 0.0001$, interaction between treatments and genotypes; *indicates differences between VEH and treated (TAM or E2) mice within each genotype. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Abbreviation: HOMA-IR, homeostasis model assessment of insulin resistance.

TABLE 1. INFLUENCE OF TAM AND E2 ON PLASMA METABOLIC PARAMETERS IN HFD-FED *LERKO* MICE

	WT			*LERKO*		
	VEH	TAM	E2	VEH	TAM	E2
ALT, U/L	36 ± 8	23 ± 9	15 ± 10	28 ± 5	25 ± 20	28 ± 19
Chol, mmol/L	3.20 ± 0.20	2.07 ± 0.33†	3.23 ± 0.64	3.57 ± 0.35	3.55 ± 0.22	3.21 ± 0.23
HDL-C, mmol/L	1.67 ± 0.05	1.23 ± 0.08‡	1.32 ± 0.12*	1.86 ± 0.21	1.83± 0.14	1.34 ± 0.16†
LDL-C, mmol/L	0.35 ± 0.03	0.43 ± 0.04*	0.77 ± 0.25*	0.47 ± 0.05	0.39 ± 0.04*	0.64 ± 0.15*
TG, mmol/L	0.88 ± 0.05	0.82 ± 0.06	0.77 ± 0.16	0.87 ± 0.09	0.98 ± 0.20	0.71 ± 0.05*
FFA, mmol/L	0.77 ± 0.11	0.93 ± 0.14	0.63 ± 0.14	1.07 ± 0.14	0.87 ± 0.07*	0.78 ± 0.17*
Adiponectin, ng/mL	6.7 ± 1.0	4.7 ± 0.4†	2.8 ± 0.5†	7.1 ± 0.9	4.2 ± 0.3‡	3.1 ± 0.6‡
Leptin, ng/mL	20.3 ± 4.8	3.8 ± 1.1‡	2.1 ± 1.1‡	23.8 ± 4.7	19.1 ± 3.4	3.3 ± 2.3‡
Resistin, ng/mL	5.2 ± 0.9	3.1 ± 0.8†	5.0 ± 1.6	4.8 ± 1.7	3.6 ± 0.7	3.9 ± 0.6

Ovariectomized female *LERKO* mice and their respective WT littermates were fed an HFD and concomitantly treated with TAM, E2, or VEH for 12 weeks. Blood samples were collected at death in 3-hour-fasted mice. Data are expressed as mean ± SEM.
Differences between VEH-treated and either TAM- or E2-treated mice according to the Student *t* test; $^*P < 0.05$; $^\dagger P < 0.01$; $^\ddagger P < 0.001$.
Abbreviations: ALT, alanine aminotransferase; C, cholesterol; Chol, total cholesterol; FFA, free fatty acids; TG, triglyceride.

physical activity (Fig. 3C) in WT mice along with a significant reduction in energy expenditure during both day and night periods (Fig. 3B). TAM-treated WT mice were also characterized by a reduction in oxygen consumption and carbon dioxide production (Supporting Fig. S3) that was associated with a significant decrease in food intake (Fig. 2B), thus suggesting that the metabolic benefit conferred by TAM probably results in a large part from its anorectic effect. Finally, all the effects of TAM on food intake (Fig. 2B) and energy expenditure (Fig. 3B) were entirely abrogated in *LERKO* mice, demonstrating that they are mediated through ERα activation in hepatocytes.

TAM ENHANCES *Gdf15* EXPRESSION THROUGH HEPATOCYTE ERα ACTIVATION

To identify the link between hepatocyte ERα activation and whole-body metabolic protection conferred by TAM, we first explored whether TAM and E2 differently influence the expression of hepatokines known to exert specific actions on energy and glucose homeostasis. Neither E2 nor TAM altered angiopoietin-like 4 (*Angptl4*), *Angptl6*, insulin-like growth factor 1 (*Igf1*), and insulin-like growth factor binding protein 1 (*Igfbp1*) hepatic expression levels in WT mice, whereas *Angptl4 and Igfbp1* were significantly increased in E2-treated *LERKO* mice (Fig. 4A). However, E2 enhanced fibroblast growth factor 21 (*Fgf21*) mRNA expression level in the liver from WT mice (Fig. 4A), as reported,[14] and this positive regulation was still observed in *LERKO* mice (Fig. 4A). Similarly, E2 induced a significant upregulation of energy homeostasis associated (*Enho*) that codes for adropin, a hepatokine known to promote nocturnal physical activity and to prevent insulin resistance and adiposity, independently of hepatocyte ERα activation (Fig. 4A). TAM significantly increased the expression of *Igfbp2* in WT but not *LERKO* mice (Fig. 4A). The plasma level of this hepatokine has been demonstrated to be inversely correlated with incidence of type 2 diabetes. However, the strongest

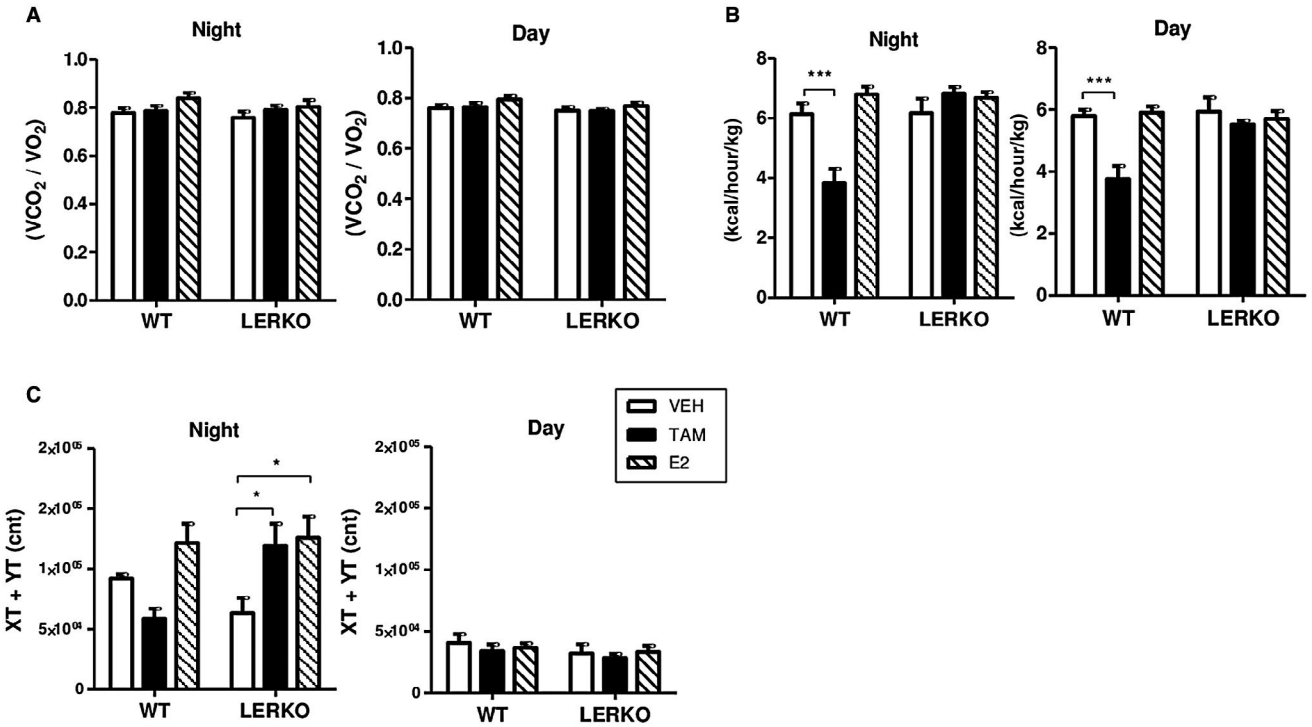


FIG. 3. Effects of TAM and E2 and role of hepatocyte ERα on energy homeostasis. Indirect calorimetry measurements were performed in *LERKO* mice (n = 19) and their respective WT littermates (n = 21) fed an HFD and treated with TAM, E2, or VEH for 12 weeks. (A) Respiratory exchange ratio calculated as carbon dioxide production/oxygen consumption, (B) energy expenditure (kcal heat produced). (C) Ambulatory physical activity as measured by the average number of beambreak counts in different dimensions (XT + YT). Data are expressed as mean ± SEM. *indicates differences between VEH and treated (TAM or E2) mice within each genotype. $^{*}P < 0.05$; $^{***}P < 0.001$. Abbreviations: VCO_2, carbon dioxide production; VO_2, oxygen consumption.

effect of TAM on gene expression concerned *Gdf15*, which codes for a molecule described as a powerful cytokine that inhibits food intake and decreases body weight through direct action on feeding centers in the hypothalamus and brainstem.[15,16] Indeed, an 8-fold increase in *Gdf15* mRNA hepatic expression was observed in TAM-treated WT mice, contrasting with the lack of E2 effect on this hepatokine expression (Fig. 4A). TAM-mediated up-regulation of *Gdf15* mRNA hepatic expression was totally abolished in *LERKO* mice (Fig. 4A) and, importantly, serum Gdf15 concentrations were perfectly correlated with its liver expression profile in WT as in *LERKO* mice (Fig. 4B). We also determined *Gdf15* mRNA levels in adipose tissue from WT and *LERKO* mice and found no influence of E2 and only a slight increase in TAM-treated mice from both genotypes (Fig. 4C), supporting the conclusion that serum Gdf15 concentration mainly reflects liver secretion. Our data thus demonstrate that unlike E2, TAM administration induces a marked increase in *Gdf15* hepatic expression and Gdf15 circulating concentration through the activation of ERα in hepatocytes.

DIFFERENTIAL REGULATION OF *Gdf15* EXPRESSION IN THE LIVER WITH TAM AND E2

To further explore the molecular mechanisms involved in the differential regulation of *Gdf15* expression by both ERα ligands, ovariectomized female mice were treated with E2 or TAM either acutely (single subcutaneous injection) or chronically (through subcutaneous pellet over 3 weeks). After acute treatment, E2 and TAM up-regulated *Gdf15* expression at the same level (Fig. 5A), in agreement with our previous study emphasizing *Gdf15* mRNA induction in the livers of female mice following oral

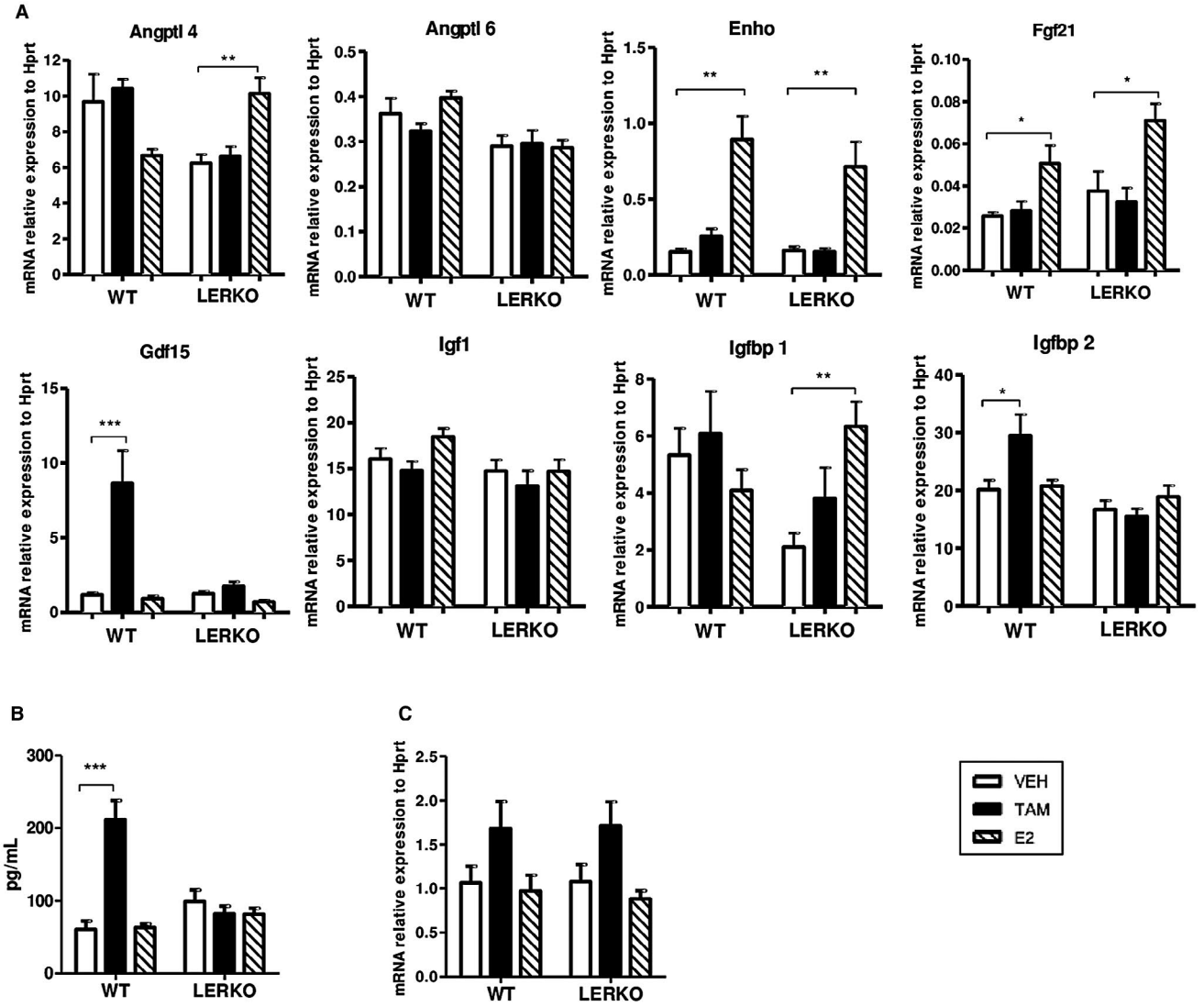


FIG. 4. Role of hepatocyte ERα to TAM or E2 regulation of hepatokines known to improve glucose and lipid homeostasis and/or to control food intake. *LERKO* mice (n = 18) and their respective WT littermates (n = 17) were fed an HFD and treated with TAM, E2, or VEH for 12 weeks. (A) Hepatic mRNA expression of hepatokines (*Angptl4, Angptl6, Enho, Fgf21, Gdf15, Igf1, Igfbp1,* and *Igfbp2*) in liver tissue samples. (B) Serum Gdf15 concentration in blood samples. (C) *Gdf15* mRNA expression in white adipose tissues. Data are expressed as mean ± SEM. *indicates differences between VEH and treated (TAM or E2) mice within each genotype. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Abbreviation: Hprt, hypoxanthine phosphoribosyl transferase.

E2 administration.[17] However, no *Gdf15* mRNA level induction was observed after E2 chronic treatment (Fig. 5A), as shown in HFD-fed mice (Fig. 4A). By contrast, TAM chronic treatment still increased *Gdf15* expression. This was not due to ERα protein degradation in response to E2 because neither E2 nor TAM chronic treatment altered ERα protein level (Fig. 5B). In ChIP-quantitative polymerase chain reaction (qPCR) experiments, ERα and its coactivator CREB binding protein (CBP) were found to be associated to their binding sites (BS1 and BS2) in the *Gdf15* regulatory region[17] in response to TAM but not to E2 chronic administration (Fig. 5C; Supporting Fig. S6A). In liver from E2-treated mice, the lack of ERα binding to the *Gdf15* regulatory region was associated with trimethylation of the twenty-seventh amino acid in histone H3 (H3K27) on BS1 (Fig. 5D; Supporting Fig. S6B), an epigenetic modification recently described to silence *Gdf15* expression.[18] These ChIP assays also demonstrated

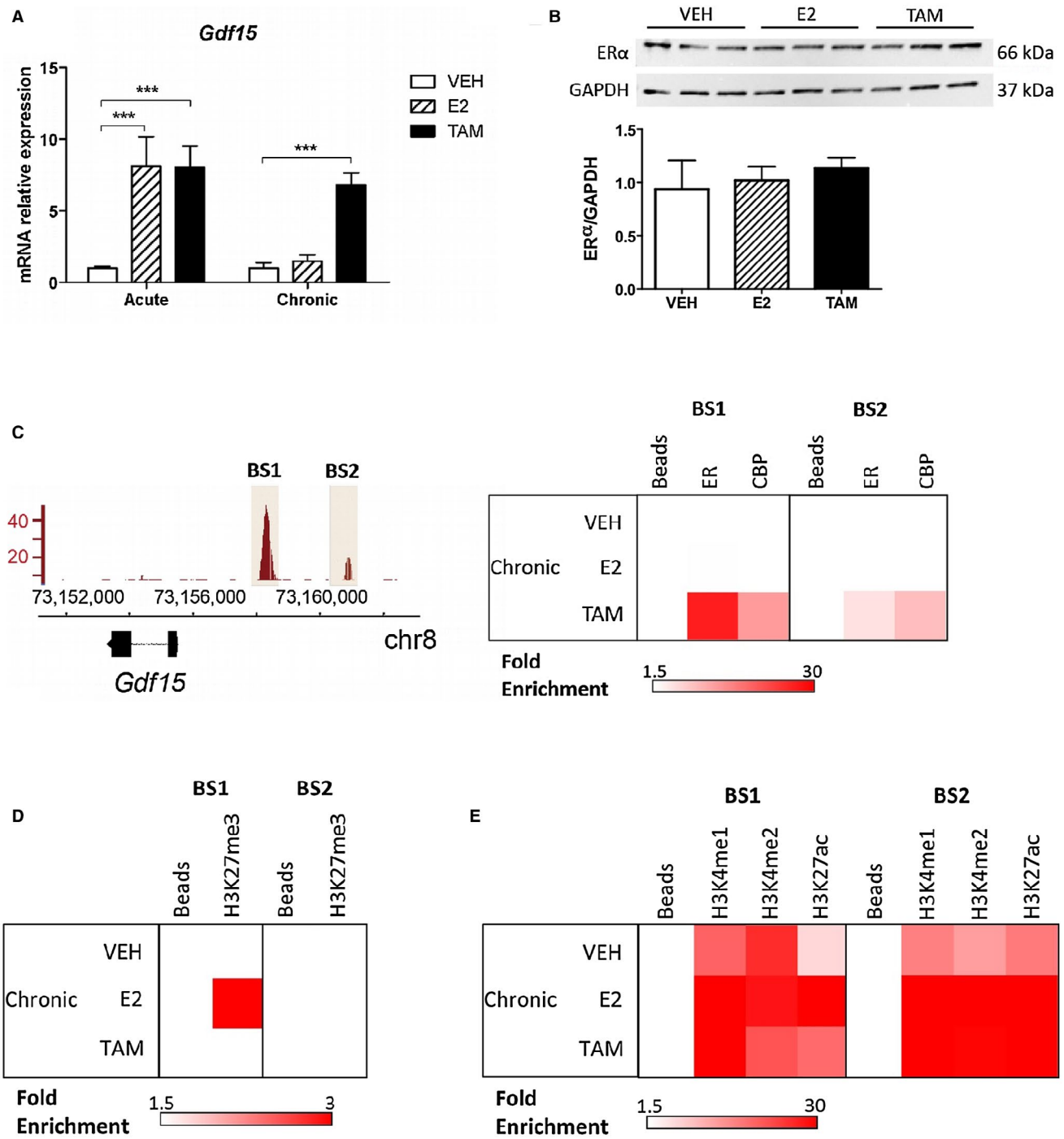


FIG. 5. Regulation of *Gdf15* expression in the liver with TAM and E2. Ovariectomized female *C57Bl/6J* mice (n = 12) were treated with TAM, E2, or VEH for 2 hours (acute) or for 3 weeks (chronic). (A) Hepatic *Gdf15* mRNA expression was determined by RT-qPCR in liver tissue samples. Data are expressed as mean ± SEM. ***$P < 0.001$. (B) Hepatic ERα protein level after chronic treatment was determined by western blot and normalized to Gapdh. (C) ERα enrichment at BS1 and BS2 on the *Gdf15* promoter region (left panel) was quantified by ChIP-qPCR experiments from livers treated as in panel B (right panel). The presence of histone marks (D) H3K27me3 and (E) H3K4me1, H3K4me2, and H3K27ac on BS1 and BS2 were assessed by ChIP-qPCR experiments on chromatin prepared from the same livers as those used in panel C. Abbreviation: chr, chromosome; Gapdh, glyceraldehyde 3-phosphate dehydrogenase; RT, reverse transcription.

that the nucleosomes located around both BS retained acetylated H3K27 (H3K27ac) and H3K4 monomethylation (me1) and dimethylation chromatin marks (Fig. 5E). Interestingly, both BS1 and BS2 still exhibited an enhanced enrichment in H3K4me1 and H3K27ac marks in the presence of E2 compared to VEH (Supporting Fig. S6C). This is consistent with the hypothesis that these sequences were likely to have undergone activation at an earlier time point in accordance with the E2-mediated induction of *Gdf15* observed in the acute condition. Altogether, these data fit the hypothesis that at least BS1 begins to acquire features from "poised" enhancers, i.e., genomic sites that were active and underwent relative transient inactivation.[19] Finally, the chronic administration of TAM appears to be less efficient than E2 in inducing the enrichment of both BS1 and BS2 in H3K4me1 and H3K27ac (Supporting Fig. S6C). This may reflect the weaker ability of ERα to recruit cofactors in the presence of TAM compared to E2, which in this precise case does not prevent ERα from modulating the transcription rate of *Gdf15*.

HEPATIC AND WHOLE-BODY METABOLIC PROTECTIVE EFFECTS OF TAM ARE ABOLISHED IN HFD-FED *GDF15KO* MICE

To determine whether Gdf15 contributes to the overall metabolic protective actions of TAM, ovariectomized *GDF15KO* mice and their WT littermates were treated with TAM, E2, or VEH for 6 weeks and concomitantly fed an HFD. The protective actions of E2 observed in WT mice in terms of body weight gain, fat mass accumulation, glucose tolerance, and steatosis were not altered by *Gdf15* deficiency (Fig. 6A-E). In striking contrast, TAM failed to protect *GDF15KO* mice from HFD-induced obesity (Fig. 6A,B), glucose intolerance (Fig. 6D), and steatosis (Fig. 6E). In addition, the anorectic effect of TAM was abolished and an increase in food intake was even observed in TAM-treated *GDF15KO* mice (Fig. 6C). Altogether, our results demonstrate that the selective modulation of hepatocyte ERα with TAM preserves whole-body energy and glucose homeostasis, in a context of HFD feeding, through the up-regulation of *Gdf15* hepatic expression.

Discussion

The present study further emphasizes the protective role of hepatocyte-specific ERα signaling against diet-induced steatosis. Noteworthy, in our experimental settings, such a beneficial effect on the liver was not only observed with E2 administration but also and to a similar extent following TAM treatment. This latter observation is undoubtedly more surprising because numerous clinical observations reported that TAM is able to promote or to worsen fatty liver in women prescribed with this treatment for breast cancer, although recent studies concluded that the incidence of TAM-induced steatosis is very low.[20] Studies conducted in rodent models led to divergent results depending on the experimental settings, some of them reproducing TAM-induced steatosis[21,22] while others, in perfect agreement with our observations, reported protective effects of TAM on the liver[23,24] but also on glucose and lipid metabolism.[24-28] Accordingly, Ceasrine et al.[24] highlighted the need for caution in the interpretation of metabolic studies that require transient high-dose TAM administration protocols for creating conditional KO in tamoxifen-inducible estrogen receptor (*ERT2*)-*Cre* thyroglobulin (*Tg*) mice, demonstrating an improved glucose tolerance in both male and female mice 1 week after the last dose and even 3 weeks later in male mice.

However, our main finding is that hepatocyte ERα, following the peculiar mode of activation of TAM, is also able to preserve whole-body energy and glucose homeostasis from the deleterious effects of HFD feeding. Indeed, hepatocyte-restricted ERα deletion differently alters the metabolic protection conferred by E2 and TAM, two ERα ligands respectively leading to the full activation or to the AF1-selective modulation of the receptor.[6,8] Although E2-treated *LERKO* female mice were no longer protected from steatosis, as reported,[4] these mice were still preserved from HFD-induced adiposity, insulin resistance, and glucose intolerance, underlining the involvement of targets other than hepatocytes for these latter actions of E2. In contrast, similar extensive protective actions exerted by TAM are dependent on hepatocyte ERα activation as they were all abolished in *LERKO* mice. Consequently, although TAM and E2 similarly protect female mice from HFD-induced metabolic disturbances through ERα-dependent mechanisms, our results support the conclusion that their respective effects involve distinct cellular targets.

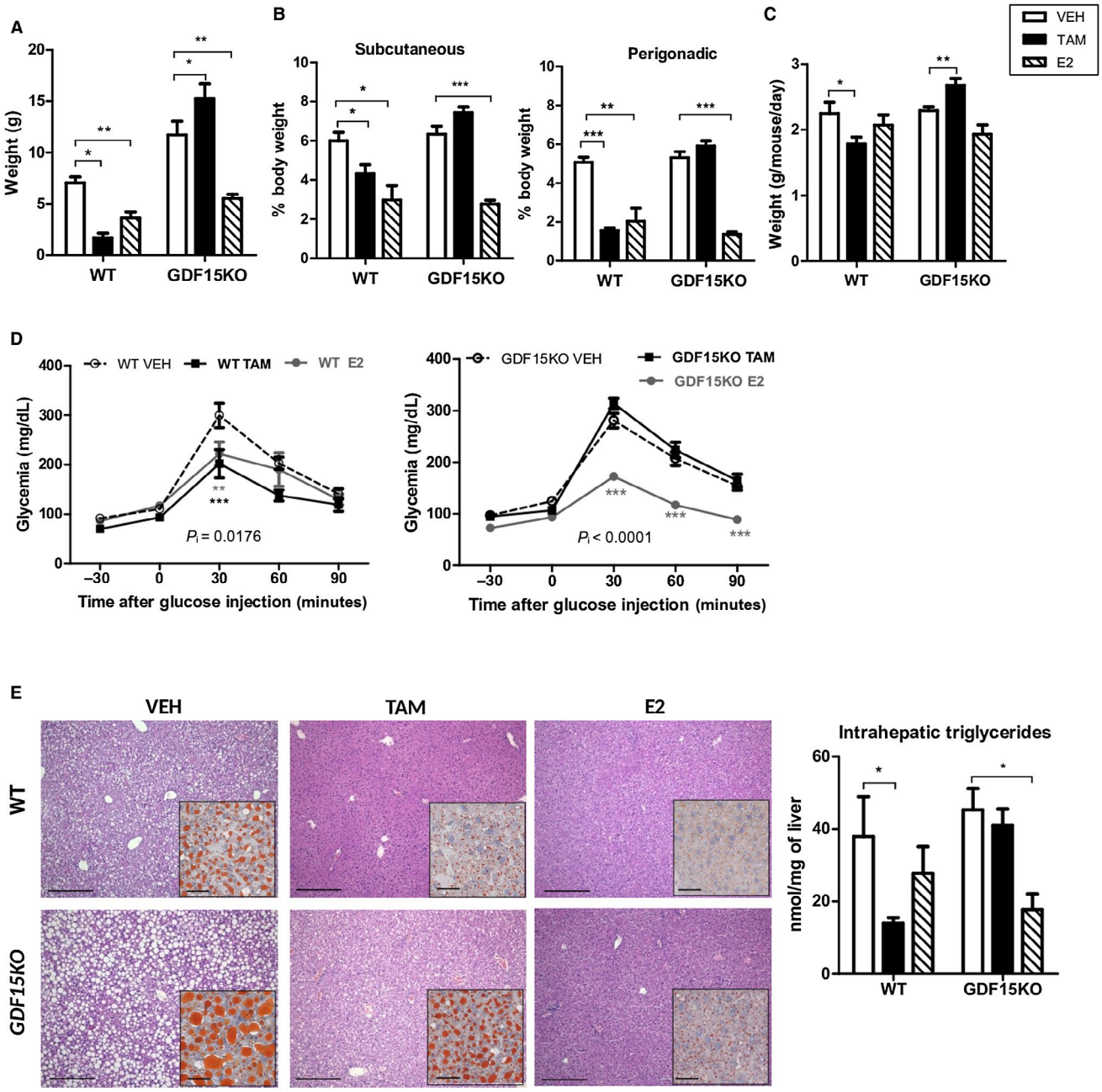


FIG. 6. Protective metabolic effects of TAM are abolished in *GDF15KO* mice. *GDF15KO* mice (n = 23) and their respective WT littermates (n = 14) were fed an HFD and treated with TAM, E2, or VEH for 6 weeks. (A) Body weight gain; (B) food intake; (C) adipose tissue distribution; (D) intraperitoneal glucose tolerance tests; (E) liver histology, representative images of H&E (scale bars, 100 μm) and Oil Red O (insets; scale bars, 50 μm) staining and intrahepatic triglyceride content. Data are expressed as mean ± SEM. P_i, interaction between treatments and genotypes. *indicates differences between VEH and treated (TAM or E2) mice. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

It is now well recognized that the overall beneficial metabolic action of E2 requires ERα activation in various tissues, including the central nervous system, adipose tissue, liver, and endocrine pancreas.[29] In particular, estrogens have been demonstrated to regulate different aspects of energy homeostasis through direct activation of ERα in specific types of neurons.[30] ERα deletion restricted to hypothalamic

steroidogenic factor-1 neurons induces hypometabolism and abdominal obesity without associated hyperphagia, whereas ERα activation in hypothalamic pro-opiomelanocortin neurons controls food intake without any direct influence on energy expenditure or fat distribution.[30]

In our experimental settings, E2 administration enhanced nocturnal physical activity level in both WT and *LERKO* female mice while TAM lowered energy expenditure in WT mice, probably resulting from a significant decrease in food intake as described in murine models.[25,31-33] Interestingly, although TAM anorectic effect has been attributed to Fasn inhibition in the ventromedial nucleus of the hypothalamus, leading to accumulation of malonyl-CoA,[31] no study has demonstrated that this action relies on the direct activation of ERα in hypothalamic neurons. Noteworthy, demonstrating that the influence of TAM on food intake and energy expenditure was totally abrogated in *LERKO* mice, the present data suggest that the control of these central regulations by TAM mainly results from peripheral signals induced by ERα selective modulation in hepatocytes.

For insight into the mechanisms leading to the discrepant metabolic consequences of hepatocyte ERα activation by E2 and TAM, we addressed their respective influence on hepatic gene expression, first focusing on a set of genes involved in the main metabolic pathways. In line with their similar protective effects against HFD-induced steatosis, both molecules down-regulated the transcription of genes involved in *de novo* lipogenesis and lipid synthesis, although with a wider influence of TAM that more specifically impacted some of them (*Cebpa*, *Nr1h3*, *Pemt*) in WT mice. E2 and TAM also differently alter the expression of some genes involved in lipid and/or glucose metabolism (*Apoa1*, *ApoA4*, *Cyp17a1*, *Lepr*). However, the most important observation is that TAM-induced gene regulations were all abolished in *LERKO* mice whereas E2 still influenced the expression of several liver genes in these animals, thus probably through indirect mechanisms mediated by other ERα-expressing cellular targets that remain to be identified.

We then hypothesized that the whole-body protection conferred by TAM through hepatocyte ERα activation could be mediated by liver-secreted circulating factors, and we decided to focus our analyses on the respective influence of E2 and TAM on the expression of hepatokines described to exert metabolic actions. It must be acknowledged that additional experiments would have been useful to definitely demonstrate that TAM acts through hepatocyte-initiated endocrine mechanisms. From our data, we cannot exclude that neurovegetative signals and other liver-derived factors, including other proteins or small molecules, could contribute to the systemic effects of TAM. Importantly, hepatocyte ERα is already known as a key mediator of systemic regulation as it controls *Igf1* expression.[9] In our experiments, E2 treatment led to a significant increase in hepatic expression of *Fgf21*, as reported,[14] and also of *Enho*, which codes for adropin, an hepatokine known to increase night physical activity and to prevent insulin resistance and adiposity.[34] These regulations are demonstrated to be totally independent from hepatocyte ERα and could thus have contributed, at least partially, to the preservation of energy and glucose homeostasis by E2 in HFD-fed WT and *LERKO* mice. Conversely, neither *Fgf21*, in line with the action of another SERM (bazedoxifene),[14] nor *Enho* expression was modified in TAM-treated mice livers. Among the genes coding for hepatokines tested in our experimental settings, we found that *Gdf15* and, to a much lesser extent *Igfbp2*, displays a gene expression profile strictly dependent on TAM-induced hepatocyte ERα activation.

Noteworthy, *Gdf15* gene expression in the liver perfectly correlates with plasma concentrations measured in WT and *LERKO* mice. Mainly produced by the liver,[35] Gdf15 is a divergent member of the transforming growth factor-β superfamily, which exerts anorectic effects and controls body weight through the activation of the glial cell line-derived neurotrophic factor family receptor α-like (GFRAL), recently identified as the specific receptor of Gdf15 in hindbrain neurons of the area postrema and nucleus of the solitary tract.[36-38] In rodent models, even a moderate increase in Gdf15 circulating levels reduces food intake, and a sustained exposition to this hepatokine leads to cachexia, both under normal diet and HFD.[15,39] Accordingly, *GDF15KO* mice are characterized by accelerated weight gain and increased fat mass, mainly localized in visceral abdominal sites, but their food intake and body weight returned to the WT level following infusion with recombinant human GDF15.[40] Moreover, *GDF15KO* mice are also prone to develop steatosis, while *GDF15*-transgenic mice are protected from steatosis and associated metabolic disorders.[41]

Finally, in agreement with our results, prevention of obesity in HFD-fed mice treated with recombinant GDF15 was not associated with an increase in energy expenditure, suggesting that the protective action of this hepatokine is likely driven by the reduction in food intake.[36] These considerations prompted us to directly address the role of Gdf15 in whole-body metabolic protective TAM effects. The effects of TAM were confirmed in *GDF15*$^{+/+}$ female mice but were all totally abrogated in *GDF15KO* mice.

Although hepatocytes have been recognized as the main cell sources of Gdf15 in the liver,[42] whether TAM-enhanced *Gdf15* expression is restricted to hepatocytes or concerns other cell types remains to be determined. However, this lack of demonstration of the cellular sources of Gdf15 in the liver of TAM-treated mice does not challenge our main conclusion, i.e., the specific regulation of liver *Gdf15* by TAM requires ERα activation in hepatocytes. *GDF15* expression levels are physiologically low but rapidly increase in pathophysiological conditions, such as tissue injury, inflammation, and malignancy, as well as during dysmetabolic status, including obesity, type 2 diabetes, and NAFLD.[41] Interestingly, our study demonstrates that hepatocyte ERα is also able to enhance *Gdf15* expression according to its mode of activation. Contrasting with the significant increase induced by TAM, *Gdf15* liver expression and Gdf15 circulating levels were not altered by E2 chronic treatment. This latter observation is surprising because we previously observed and confirmed in the present study that acute E2 administration significantly increases liver *Gdf15* gene transcription.[17] The discrepancy in Gdf15 transcription regulation following chronic treatments cannot be explained by ERα protein degradation in response to E2, but ChIP-qPCR revealed that ERα and its coactivator CBP were bound to BS1 and BS2 in the Gdf15 regulatory region only in TAM-treated mice. Accordingly, our data suggest that chronic E2 treatment induced epigenetic modifications that contribute to silent Gdf15 expression, as recently reported.[18]

In line with our recent work,[6,8] the present study further illustrates the redundancy between ERα-AF1 and ERα-AF2 in terms of metabolic protection. In contrast, both ERα-AFs are required to mediate proliferative effects on the mammary gland and contribute to the increased risk of breast cancer elicited by estrogens, contrasting with the spectacular protection conferred by ERα-AF2 antagonism of TAM.[43] However, this ERα-AF2 antagonism has no adverse impact on the main cellular target(s), mainly hepatocytes as demonstrated here, involved in TAM metabolic effects. This underlines the therapeutic potential of SERMs resulting in the specific activation of only ERα-AF1. Furthermore, as discussed above, the cellular targets mediating the metabolic protective actions are different according to the nature of the ligand, although leading to a similar overall benefit.

From a therapeutic perspective, it would be very interesting to design new strategies for a preferential delivery of ERα-AF1-selective modulators to the liver, as recently proposed for targeting other cellular types, such as the pancreas with a glucagon-like peptide-1-estrogen conjugate.[44]

The present work provides further evidence that hepatocyte ERα represents a promising target for the prevention of NAFLD and demonstrates for the first time that the selective modulation of ERα in hepatocytes is sufficient to preserve whole-body energy and glucose homeostasis. The mechanism accounting for this major widespread metabolic action relies on the induction of the hepatokine Gdf15. Selective modulation of hepatocyte ERα leading to sustained increase in circulating Gdf15 could thus be sufficient to fight obesity and its related complications, such as type 2 diabetes and NAFLD.

Acknowledgment: We acknowledge Marc Poirot, Henrik Laurell, Alexia Vinel, Sandra Handgraaf, Marine Adlanmerini, and Professor Walter Wahli for their help in the critical interpretation of data. We thank Professor Pierre Chambon (Strasbourg, France) and Dr. Björn Spittau (Freiburg, Germany) for providing floxed *ERα* and *GDF15KO* mice, respectively. We also acknowledge the skillful technical assistance of Frédéric Boudou, Adrien Pulido, the histology platform of Institut National Polytechnique de Toulouse (INP)-École Nationale Vétérinaire de Toulouse (Isabelle Bleuart and Isabelle Pardo), the Institut National de la Santé et de le Recherche Médicale (INSERM) Unité Médicale de Recherche (UMR) 1048 platforms (Toulouse, France) ANEXPLO-GENOTOUL (Cédric Baudelin, Aurore Desquesnes, Sophie Le Gonidec), GeT-TQ_ Genopole (Frédéric Martins, Jean-José Maoret), MetaToul-LIPIDOMIQUE (Justine Bertrand-Michel), and the Institut National de la Santé et de le Recherche Médicale (INSERM) histology platform (Lucie Fontaine).

REFERENCES

1) Hevener AL, Clegg DJ, Mauvais-Jarvis F. Impaired estrogen receptor action in the pathogenesis of the metabolic syndrome. Mol Cell Endocrinol 2015;418:306-321.
2) Valera MC, Fontaine C, Dupuis M, Noirrit-Esclassan E, Vinel A, Guillaume M, et al. Towards optimization of estrogen receptor modulation in medicine. Pharmacol Ther 2018;189:123-129.
3) Wang X, Lu Y, Wang E, Zhang Z, Xiong X, Zhang H, et al. Hepatic estrogen receptor alpha improves hepatosteatosis through upregulation of small heterodimer partner. J Hepatol 2015;63:183-190.
4) Zhu L, Brown WC, Cai Q, Krust A, Chambon P, McGuinness OP, et al. Estrogen treatment after ovariectomy protects against fatty liver and may improve pathway-selective insulin resistance. Diabetes 2013;62:424-434.
5) Smith CL, O'Malley BW. Coregulator function: a key to understanding tissue specificity of selective receptor modulators. Endocr Rev 2004;25:45-71.
6) **Handgraaf S**, **Riant E**, Fabre A, Waget A, Burcelin R, Liere P, et al. Prevention of obesity and insulin resistance by estrogens requires ERalpha activation function-2 (ERalphaAF-2), whereas ERalphaAF-1 is dispensable. Diabetes 2013;62:4098-4108.
7) Berry M, Metzger D, Chambon P. Role of the two activating domains of the oestrogen receptor in the cell-type and promoter-context dependent agonistic activity of the anti-oestrogen 4-hydroxytamoxifen. EMBO J 1990;9:2811-2818.
8) Guillaume M, Handgraaf S, Fabre A, Raymond-Letron I, Riant E, Montagner A, et al. Selective activation of estrogen receptor alpha activation function-1 is sufficient to prevent obesity, steatosis, and insulin resistance in mouse. Am J Pathol 2017;187:1273-1287.
9) Della Torre S, Rando G, Meda C, Stell A, Chambon P, Krust A, et al. Amino acid-dependent activation of liver estrogen receptor alpha integrates metabolic and reproductive functions via IGF-1. Cell Metab 2011;13:205-214.
10) Strelau J, Strzelczyk A, Rusu P, Bendner G, Wiese S, Diella F, et al. Progressive postnatal motoneuron loss in mice lacking GDF-15. J Neurosci 2009;29:13640-13648.
11) Matic M, Bryzgalova G, Gao H, Antonson P, Humire P, Omoto Y, et al. Estrogen signalling and the metabolic syndrome: targeting the hepatic estrogen receptor alpha action. PLoS ONE 2013;8:e57458.
12) Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911-917.
13) Barrans A, Collet X, Barbaras R, Jaspard B, Manent J, Vieu C, et al. Hepatic lipase induces the formation of pre-beta 1 high density lipoprotein (HDL) from triacylglycerol-rich HDL2. A study comparing liver perfusion to in vitro incubation with lipases. J Biol Chem 1994;269:11572-11577.
14) Kim JH, Meyers MS, Khuder SS, Abdallah SL, Muturi HT, Russo L, et al. Tissue-selective estrogen complexes with bazedoxifene prevent metabolic dysfunction in female mice. Mol Metab 2014;3:177-190.
15) Johnen H, Lin S, Kuffner T, Brown DA, Tsai VW, Bauskin AR, et al. Tumor-induced anorexia and weight loss are mediated by the TGF-beta superfamily cytokine MIC-1. Nat Med 2007;13:1333-1340.
16) Tsai VW, Manandhar R, Jorgensen SB, Lee-Ng KK, Zhang HP, Marquis CP, et al. The anorectic actions of the TGFbeta cytokine MIC-1/GDF15 require an intact brainstem area postrema and nucleus of the solitary tract. PLoS ONE 2014;9:e100370.
17) Palierne G, Fabre A, Solinhac R, Le Peron C, Avner S, Lenfant F, et al. Changes in gene expression and estrogen receptor cistrome in mouse liver upon acute E2 treatment. Mol Endocrinol 2016;30:709-732.
18) Lu X, He X, Su J, Wang J, Liu X, Xu K, et al. EZH2-mediated epigenetic suppression of GDF15 predicts a poor prognosis and regulates cell proliferation in non-small-cell lung cancer. Mol Ther Nucleic Acids 2018;12:309-318.
19) Rada-Iglesias A, Bajpai R, Swigut T, Brugmann SA, Flynn RA, Wysocka J. A unique chromatin signature uncovers early developmental enhancers in humans. Nature 2011;470:279-283.
20) Bruno S, Maisonneuve P, Castellana P, Rotmensz N, Rossi S, Maggioni M, et al. Incidence and risk factors for non-alcoholic steatohepatitis: prospective study of 5408 women enrolled in Italian tamoxifen chemoprevention trial. BMJ 2005;330:932.
21) Cole LK, Jacobs RL, Vance DE. Tamoxifen induces triacylglycerol accumulation in the mouse liver by activation of fatty acid synthesis. Hepatology 2010;52:1258-1265.
22) Lelliott CJ, Lopez M, Curtis RK, Parker N, Laudes M, Yeo G, et al. Transcript and metabolite analysis of the effects of tamoxifen in rat liver reveals inhibition of fatty acid synthesis in the presence of hepatic steatosis. FASEB J 2005;19:1108-1119.
23) Miyashita T, Toyoda Y, Tsuneyama K, Fukami T, Nakajima M, Yokoi T. Hepatoprotective effect of tamoxifen on steatosis and non-alcoholic steatohepatitis in mouse models. J Toxicol Sci 2012;37:931-942.
24) Ceasrine AM, Ruiz-Otero N, Lin EE, Lumelsky DN, Boehm ED, Kuruvilla R. Tamoxifen improves glucose tolerance in a delivery, sex, and strain-dependent manner in mice. Endocrinology 2019;160:782-790.
25) Lampert C, Arcego DM, Laureano DP, Diehl LA, da Costa Lima IF, Krolow R, et al. Effect of chronic administration of tamoxifen and/or estradiol on feeding behavior, palatable food and metabolic parameters in ovariectomized rats. Physiol Behav 2013;119:17-24.
26) Goss PE, Qi S, Hu H. Comparing the effects of atamestane, toremifene and tamoxifen alone and in combination, on bone, serum lipids and uterus in ovariectomized rats. J Steroid Biochem Mol Biol 2009;113:233-240.
27) Reckless J, Metcalfe JC, Grainger DJ. Tamoxifen decreases cholesterol sevenfold and abolishes lipid lesion development in apolipoprotein E knockout mice. Circulation 1997;95:1542-1548.
28) Ke HZ, Chen HK, Simmons HA, Qi H, Crawford DT, Pirie CM, et al. Comparative effects of droloxifene, tamoxifen, and estrogen on bone, serum cholesterol, and uterine histology in the ovariectomized rat model. Bone 1997;20:31-39.
29) Mauvais-Jarvis F, Clegg DJ, Hevener AL. The role of estrogens in control of energy balance and glucose homeostasis. Endocr Rev 2013;34:309-338.
30) Xu Y, Nedungadi TP, Zhu L, Sobhani N, Irani BG, Davis KE, et al. Distinct hypothalamic neurons mediate estrogenic effects on energy homeostasis and reproduction. Cell Metab 2011;14:453-465.
31) Lopez M, Lelliott CJ, Tovar S, Kimber W, Gallego R, Virtue S, et al. Tamoxifen-induced anorexia is associated with fatty acid synthase inhibition in the ventromedial nucleus of the hypothalamus and accumulation of malonyl-CoA. Diabetes 2006;55:1327-1336.
32) Wallen WJ, Belanger MP, Wittnich C. Sex hormones and the selective estrogen receptor modulator tamoxifen modulate weekly body weights and food intakes in adolescent and adult rats. J Nutr 2001;131:2351-2357.
33) Wade GN, Heller HW. Tamoxifen mimics the effects of estradiol on food intake, body weight, and body composition in rats. Am J Physiol 1993;264:R1219-R1223.
34) Kumar KG, Trevaskis JL, Lam DD, Sutton GM, Koza RA, Chouljenko VN, et al. Identification of adropin as a secreted factor linking dietary macronutrient intake with energy homeostasis and lipid metabolism. Cell Metab 2008;8:468-481.

35) Ding Q, Mracek T, Gonzalez-Muniesa P, Kos K, Wilding J, Trayhurn P, et al. Identification of macrophage inhibitory cytokine-1 in adipose tissue and its secretion as an adipokine by human adipocytes. Endocrinology 2009;150:1688-1696.
36) Yang L, Chang CC, Sun Z, Madsen D, Zhu H, Padkjaer SB, et al. GFRAL is the receptor for GDF15 and is required for the anti-obesity effects of the ligand. Nat Med 2017;23: 1158-1166.
37) Mullican SE, Lin-Schmidt X, Chin CN, Chavez JA, Furman JL, Armstrong AA, et al. GFRAL is the receptor for GDF15 and the ligand promotes weight loss in mice and nonhuman primates. Nat Med 2017;10:1150-1157.
38) Emmerson PJ, Wang F, Du Y, Liu Q, Pickard RT, Gonciarz MD, et al. The metabolic effects of GDF15 are mediated by the orphan receptor GFRAL. Nat Med 2017;23:1215-1219.
39) Macia L, Tsai VW, Nguyen AD, Johnen H, Kuffner T, Shi YC, et al. Macrophage inhibitory cytokine 1 (MIC-1/GDF15) decreases food intake, body weight and improves glucose tolerance in mice on normal & obesogenic diets. PLoS ONE 2012;7: e34868.
40) Tsai VW, Macia L, Johnen H, Kuffner T, Manadhar R, Jorgensen SB, et al. TGF-b superfamily cytokine MIC-1/ GDF15 is a physiological appetite and body weight regulator. PLoS ONE 2013;8:e55174.
41) Kim KH, Kim SH, Han DH, Jo YS, Lee YH, Lee MS. Growth differentiation factor 15 ameliorates nonalcoholic steatohepatitis and related metabolic disorders in mice. Sci Rep 2018;8:6789.
42) Tabula Muris Consortium; Overall coordination; Logistical coordination; Organ collection and processing; Library preparation and sequencing; Computational data analysis; Cell type annotation; Writing group; Supplemental text writing group; Principal investigators. Single-cell transcriptomics of 20 mouse organs creates a Tabula Muris. Nature 2018;562:367-372.
43) Jordan VC. Tamoxifen: a most unlikely pioneering medicine. Nat Rev Drug Discov 2003;2:205-213.
44) Finan B, Yang B, Ottaway N, Stemmer K, Muller TD, Yi CX, et al. Targeted estrogen delivery reverses the metabolic syndrome. Nat Med 2012;18:1847-1856.

Author names in bold designate shared co-first authorship.

Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1363/suppinfo.