Review

MOLECULAR METABOLISM

Sex, metabolism and health

Adriana Maggi [1,2,*], **Sara Della Torre** [1,2]

ABSTRACT

Background: Epidemiological and clinical studies have largely demonstrated major differences in the prevalence of metabolic disorders in males and females, but the biological cause of these dissimilarities remain to be elucidated. Mammals are characterized by a major change in reproductive strategies and it is conceivable that these changes subjected females to a significant evolutionary pressure that perfected the coupling between energy metabolism and reproduction.

Scope of review: This review will address the plausibility that female liver functions diverged significantly from males given the role of liver in the control of metabolism. Indeed, it is well known that the liver is sexually dimorphic, and this might be relevant to explain the lower susceptibility to hepatic diseases and liver-derived metabolic disturbances (such as the cardiovascular diseases) characteristic of females during their fertile period. Furthermore, estrogens and the hepatic ERα play a significant role in liver sexual-specific functions and in the control of metabolic functions.

Conclusions: A better grasp of the role of male and female sex steroids in the liver of the two sexes may therefore represent an important element to conceive novel treatments aimed at preventing metabolic diseases particularly in ageing women or limiting undesired side effect in the treatment of gender dysphoria.

Keywords Sex; Health

1. SHINING LIGHT ON THE ACTION OF ESTROGENS

Among sex steroid hormones, estrogens distinguish themselves for the variety of their target tissues. Indeed, in mammals, receptors for estrogens are present in most cells, thus enabling these hormones to regulate or interfere with a significant number of metabolic pathways. Presently, we know two isoforms of estrogen receptors (ERα and ERβ) that are present in mammals and share with the other members of the nuclear receptors (NRs) family a well conserved structural organization and mode of action [1,2]. These intracellular receptors are ligand-operated transcription factors that, in addition to their genomic actions, may interact with other cytoplasmic second messengers and nuclear proteins modulating their activities. To regulate the transcription of their target genes, ERs need to be "activated" by the cognate ligand or post-translational modifications (unliganded activation) that induce a rearrangement of the receptor structure resulting in the release of the inhibitory proteins and exposure of the receptor sequences able to recognize the "estrogen responsive elements" in the proximity of the estrogen-responsive genes [3]. Upon binding the EREs, the ERs need to interact with co-regulator proteins in order to induce or repress target gene transcription [4]. Besides enabling ERs to interact with the transcription machinery, the interaction with the co-regulators enables ERs and cognate ligands to better select the target promoters and thus acquire a significant specificity of action. This was underlined by the study of the effects of estrogenic compounds such a tamoxifen, Raloxifene, and others that, upon binding, were shown to induce an ER conformation able to interact with a subclass of co-regulators and therefore provide the receptor protein with a shade of activities ranging from partial agonist to antagonist in dependence of the tissue of action [5].

Considering the mechanism of intracellular ER action, cell response to estrogens generally occurs in the frame of hours; more recently, it was found that ERα may be palmitoylated and translocate to the cell membrane and transmit the hormonal signal by regulating G protein signaling [2]; it is plausible that this alternative type of regulation occurs in a shorter length of time (minutes). However, estrogens were reported also to induce very rapid effect (timeframe of a second or less); these were found to be mediated by a *bona fide,* membrane, G protein-coupled receptor named GPER30 [6].

Thus, estrogens may control the activities of target cells in an extremely diversified manner enabling these receptors to elicit responses extremely differentiated from cell to cell or, in the same cells, from time to time.

The molecular mechanism of estrogen action has been mostly studied in cell models; to acquire a better view on the exact ER targets and the timing of the response to estrogens in living animals, we generated a reporter mouse by integrating into the mouse genome a luciferase

[1]Center of Excellence on Neurodegenerative Diseases, University of Milan, via Balzaretti 9, 20133, Milan, Italy [2]Department of Pharmacological and Biomolecular Sciences, University of Milan, via Balzaretti 9, 20133, Milan, Italy

*Corresponding author. Center of Excellence on Neurodegenerative Diseases, University of Milan, via Balzaretti 9, 20133, Milan, Italy. Fax: +390250318375. E-mail: adriana.maggi@unimi.it (A. Maggi).

Received January 11, 2018 • Revision received February 7, 2018 • Accepted February 20, 2018 • Available online 27 February 2018

https://doi.org/10.1016/j.molmet.2018.02.012

reporter gene driven by an estrogen-responsive promoter. The reporter cassette was conceived to prevent position effects due to the site of integration and, the strict dependency of luciferase transcription by the activated ERs was fully demonstrated [7]. This mouse line, named ERE-Luc, by enabling the systematic, *spatio*-temporal analysis of estrogen action highlighted, for the first time, the liver is a major targets of estrogen action and pointed to a potential role for ER in connecting metabolic and reproductive functions [8–10]. These initial findings were later on confirmed in other laboratories [11].

2. THE INDISSOLUBLE BOND BETWEEN REPRODUCTIVE AND METABOLIC FUNCTIONS

In biology, reproduction is the key function to guarantee immortality and adaptability to living organisms. However, in an environment poor in nutrients (that characterized the evolution of current living species), an uncontrolled rate of fertility might lead to extinction. It is imaginable, therefore, that mechanisms associating reproductive functions to energy availability were established in the course of evolution; the molecular details of such reciprocal interaction were shown in insects and nematodes, but very little is known with regard this potential mutual interaction in mammals.

As mentioned, studies in the ERE-Luc mouse revealed that estrogens were very active in the liver, the metabolic organ *par excellence*. The study of the distribution of ERs in the course of phylogenesis indicated that since their appearance in the animal world (in the vertebrate Agnatha) the two organs where ERs were most expressed have been the gonads and the liver (or liver ancestor organs, like the hepatopancreas in crustaceans). This is not surprising, because, in egg laying animals, the liver is known to have a major role in the control of reproductive functions as it is primarily responsible for the synthesis of yolk proteins (vitellogenins) to the ovaries [12]. In all vertebrates, the gonadal 17β-estradiol tightly regulates such a synthesis to prevent dissipation of energies towards the synthesis of proteins not immediately required. This may explain why in all oviparous the high expression of ERs in the gonads and liver is so well conserved [10,13]. In mammals, severe malnutrition and allostatic overload reduces fertility [13,14]; considering that the mammalian egg does not require storage all the energy molecules, a characteristic of egg laying species, the question raised is whether in mammals the liver maintains its key role for the control of reproduction and to what extent the hepatic estrogen receptor is involved in this function. Actually, several lines of experimental evidence showed that the hepatic ERs participate in the control of the mammalian ovulatory cycle through the regulation of the synthesis of IGF-1 and VLDL, both necessary for a productive reproductive cycle [14,15]. Quite interesting is the fact that, at least in mice, the transcriptional activity of the hepatic ERα is under the dual control of estrogens and amino acids (AA) provided by the diet [16]. More precisely, the hepatic ERα transcriptional activity is mitigated by the lack of nutritional proteins [15], and, in the event of prolonged calorie restriction, gonadal estrogens are unable to fully activate the liver ERα and the IGF-1 and lipoproteins are no longer synthesized to the extent necessary for a successful ovulation [14]. This demonstrates that, also in mammals, nutritional signaling is relevant for fertility and that the hepatic ERs maintain the role selected by evolution consisting in its ability to regulate fertility in relation to nutrient availability. However, in spite of the major role played by the liver ERα, the fact that mice with the selective deletion of liver ERα are fertile suggests that, in mammals, this is not the only element controlling liver functions, and other endocrine factors must have acquired a role that need to be investigated.

3. THE REPRODUCTIVE STATUS AND METABOLISM IN MAMMALS

Thus, in mammals, the liver ERα maintains the role of a sensor of gonadal and nutritional cues necessary to couple fertility to the environmental conditions. However, the growth of the mammalian embryo in the mother's womb and the lactation requirement necessary for sustaining the offspring imposed a novel toll on energy metabolism that required a significant adaptation to guarantee the scrupulous use of the scarce nutrients available. As a sensor of reproductive and metabolic cues it is conceivable that the liver and hepatic ERα took over this responsibility and mechanisms were created to adjust liver ability to generate energy to the changing needs of female reproductive apparatus.

We now know that in the initial phases of pregnancy, the liver adjusts lipoprotein and cholesterol synthesis to provide the placenta with the necessary supply of precursors for steroidogenesis and membrane synthesis; in the meantime, this organ facilitates lipid transport to the adipose tissue where these molecules are stocked in large amounts to ensure the appropriate energy supply all through pregnancy [17,18]. Later on, liver metabolism contributes to the increased energy demands from the placenta feeding the growing embryo by enhancing glucose output (by 16–30%) while gluconeogenesis is reduced to maintain high levels of the amino acids (AA) necessary to the final phases of the fetus growth [19,20]. After birth, the drop of circulating estrogens promotes triglyceride synthesis, and the metabolic role of liver is driven by the needs of the mammary gland with high glucose production obtained; in this phase, β-oxidation has a predominant role for the production of the ATP required by gluconeogenesis [21,22]. Thus, the liver is clearly playing a key role in diverting energy resources to meet the needs of reproduction, but the endocrine signals directing liver functions and the mechanisms involved remain to be understood.

There is no doubt that estrogens and their hepatic receptors are very relevant for the control of liver functions. It is well known that after menopause and the cessation of estrogen synthesis by the ovaries, the incidence of non-alcoholic fatty liver diseases (NAFLD) increases significantly in women, and hormone replacement therapy reduces hepatic steatosis risk and NAFLD prevalence [24–26]. Indeed, in experimental animals, ovariectomy or liver selective ablation of the estrogen receptor is associated with increased lipid synthesis and fat deposition in the liver substantiating the clinical studies [14,27], even if the use of different selective mutants of the ER showed that ERα-mediated direct transcription in non-hepatic tissues contributes to estrogen-mediated protection against hepatic steatosis (particularly in high fat diet-fed females) [28]. Other alterations of ovarian functions are associated with NAFLD and metabolic disturbances. For instance, individuals with polycystic ovarian syndrome (PCOS) that is characterized by chronic anovulation have dyslipidemia and are overweight or obese [23,29]. Several of them develop insulin resistance (IR), impaired glucose tolerance, and type 2 diabetes mellitus (T2DM). As a result, these patients are treated with the insulin sensitized metformin. However, recent clinical studies have shown that combined oral contraceptives *plus* spironolactone are more effective than metformin for symptoms of PCOS [30], suggesting that alterations in the sex hormone production by the ovaries is the predominant element in the PCOS etiology. Estrogen therapy has important beneficial effects also on the metabolic alterations of Turner syndrome (TS), a genetic disorder caused by the partial or complete absence of one of the X chromosomes in females. Women with TS are generally obese, have low lean mass and high content of visceral adipose tissue, elevated triglycerides, and high LDL/HDL *ratio*. Treatment of these patients with

estrogens leads to decreased visceral adipose tissue, increased circulating HDL, and an improvement of hepatic functions with regulation of growth and anti-apoptotic growth factors that maintain the integrity of hepatocytes and their capacity to proliferate [31,32].

4. LIVER SEXUAL DIMORPHISM, A CONSEQUENCE OF LIVER ADAPTATION TO MAMMALIAN REPRODUCTIVE FUNCTIONS

All together, these observations suggest that in female mammals liver and hepatic ERα were able to adapt to the increased requests of the reproductive apparatus and acquired novel mechanisms enabling hepatic cells to modify their metabolism in relation to the host's reproductive status. Male mammals were not subjected to such an evolutionary pressure, thus, as a logic consequence, the female liver diverged significantly in its functions from the male liver. Indeed, among all somatic organs, the liver shows the highest degree for sexual dimorphism with 72% of the genes expressed in a sexually differentiated manner (in other organs the degree of sex-dependent variability ranges between 14 and 60%) [33,34]. Not surprisingly, the major sex differences are related to lipid and steroid metabolic pathways: in comparison to males of the same age, adult fertile females have higher rates of hepatic fatty acid uptake, esterification and VLDL-TG synthesis and release, and lower FA oxidation [35–38]. In addition, the liver of cycling females has an energy partition strategy safeguarding all energies available and stocking them in deposits; this is clearly geared to protect reproduction because in case of food deprivation during pregnancy these lipids could be mobilized providing the necessary energy to the growing embryo. As an example, in case of short-term fasting, the decision to maintain lipid synthesis using amino acids as a source of fuel is the key discriminant for the hepatic metabolism of male and female mice (Della Torre et al. Cell Metabolism, *under revision*). Several animal models showed that females are protected against lipid deposition in the liver, for instance αERKO and aromatase null male mice have significant lipid infiltration in liver not observed in females [28,39,40]. However, in all these studies, the exact role of the hepatic ERα remains to be clarified [14,41,42]

In view of the major function of liver in mammalian physio-pathology, a better understanding of the sex-specific mechanisms driving food partitioning and xenobiotics metabolism is mandatory for the comprehension of the molecular bases of a large number of metabolic disorders and for the application of appropriate pharmacological/hormonal interventions. It would be also important to understand which are the determinants of liver sexual differentiation and when during development this occurs. Sexual differentiation of the brain occurs, depending on the animal species, at the end of pregnancy/few days after birth. Several groups showed that around this time of development male gonads are activated to synthesize testosterone; this sex hormone, as such or locally converted into estrogens by the presence of the enzyme aromatase, is responsible for the masculinization of the brain circuits that after puberty will control the gonadal activities and sexual behavior [43,44]. We do not know whether such a mechanism is at the bases of liver sexual differentiation. In mice, the liver expresses both estrogen and testosterone receptors at the end of pregnancy and in the first days of life; the presence of aromatase, however, is still controversial. In humans, aromatase overexpression in liver disease was first reported in fibrolamellar hepatocellular carcinoma (HCC) cells, in hepatitis, and HCC, but, in normal liver, aromatase was reported to be detected only in fetal hepatocytes and not in neonatal to adult liver [45–49]. Thus, it is conceivable that the testosterone synthesized by the embryo or neonate is responsible for epigenetic modifications that differentiate the hepatic tissues toward the "male phenotype." On the other hand, it could be postulated that the liver is not sexually differentiated at birth, but its metabolism is simply regulated by endocrine and nervous outputs from the sexually differentiated brain. Deafferentation studies indicated earlier on that brain efferent do not have a role in liver sexual differentiation. Other studies showed that the sex-dependent secretory pattern of growth hormone (GH) (more pulsatile in males than in females) might be accountable for sex-dependent liver functions of mice [50–52]. However, only a few investigations were carried out by mimicking the release of GH with the administration of exogenous GH to demonstrate the direct involvement of this hormone in liver sexual-specific activities. These studies showed, for instance, that the induction of MUP (Major Urinary Proteins) mRNA requires the pulsatile release of GH typical of males, and such a GH release inhibits the synthesis of steroid sulfate cytochrome P-450 15 beta-hydroxylase largely present in female liver, thus suggesting a role of this mechanism in liver sex-specific activities [53,54]. Several other studies showing a correlation between pattern of GH secretion and liver sex-dependent protein synthesis were carried out in mice defeminized by neonatal treatment of pups with testosterone propionate. Such treatment is very effective in changing toward the male phenotype GH pulsatile activity [55], but it cannot be ruled out that, in addition, such a treatment has a direct effect on liver inducing its sexual differentiation.

In conclusion, while it is currently believed that liver is a sexually differentiated organ, uncertainties still exist on the mechanisms responsible.

5. THE ISSUE OF LIVER DIFFERENTIATION AND FUNCTIONS IN TRANSGENDER INDIVIDUALS

The full understanding of hepatic ERα in female liver functions might be of major help for the understanding of the onset of most metabolic and inflammatory disorders associated with estrogen deficiency, as happens with menopause. It is in fact most likely that the loss of the feed-back and feed-forward mechanisms of mutual control between ovaries and the liver have as a consequence an alteration of lipid and cholesterol metabolism, deposits of fat in the liver triggering a systemic inflammatory response that is at the bases of most pathologies associated with aging (atherosclerosis, diabetes, osteoporosis, and neural disorders). By demonstrating that liver ERα has a major role in the increased incidence of these disorders in the climacterium, we could devise novel, safer, and more efficacious HRTs envisaging the use of livers elective estrogen receptor modulators. In addition, this might help in the identification of the mechanisms that provide fertile women with selective health advantages towards men.

Most recently, several authors addressed the issue of safety in the hormonal treatments related to gender dysphoria. Gender dysphoria is the distress a person experiences as a result of the sex and gender they were assigned at birth in the case when the assigned sex does not match the person's gender identity [56,57]. People who experience gender dysphoria require cross-sex hormones to induce secondary sexual characteristics of the desired sex. For females transitioning to males, testosterone is the main hormonal agent used to induce (virilization) [58]; for males transitioning to females, estrogens are used.

In recent meta-analyses of the data in the literature, testosterone use was associated with to modest increase in BMI, hemoglobin/hematocrit, and LDL-cholesterol, and a decrease in HDL-cholesterol; estrogen was associated with lower testosterone and alanine aminotransferase levels [58,59]. In light of the relevant role of liver

sex steroid hormones in the regulation of energy metabolism, we could predict that the effect of virilization may be more associated with health risks than the opposite. However, the studies so far available are all carried out in young subjects where the long term hormonal effects are most likely still clinically undetectable.

While several studies have so far focused on the role of liver in coupling hepatic metabolism to reproduction in female mammals, very little is known with regard to the role of male liver in male reproduction and on the activity of sex steroid hormones in this organ. We know that in mouse, ERα and androgen receptor (AR) are expressed in males, but the content of ERα is significantly lower than in females [12]; so far very little is known on the role of these two receptors in the regulation of energy metabolism and food partitioning in males. In this context, a better understanding would be helpful in predicting the potential harm of testosterone treatments. In addition, health status would be a priority for understanding the mechanism determining liver sex-specific functions. In case of neonatal differentiation of the liver, it would be important to understand to what extent the liver may have been masculinized in a female who is transitioning to male, because, in these individuals, the effect of testosterone treatment during adolescence might have effect much different than those in subject in which the liver has a "female phenotype." Thus, along the effects of mental health, medical, and surgical interventions on morbidity and mortality, a major priority in transgender individuals would be to determine health disparities and comorbid health conditions over the life span.

In conclusion, the understanding of liver ERα activities in female liver opens new perspectives for the understanding of the sex-dependent prevalence of several pathologies associated with aging and should be of inspiration for further studies aimed in particular to identify efficacious treatments for metabolic disorders in both sexes.

FUNDING

This work was supported by grants from the European Community ERC-2012 (ERC-Advanced Grant Ways, 322977), Seventh Framework Programme (http://www.sciencedirect.com/science/article/pii/S1550413117300414?via%3DihubFP7/2007–2013) under grant agreement no. 278850 (INMiND), and CARIPLO Foundation (grant 2013–0786).

AUTHOR CONTRIBUTION

A.M. and S.D.T. equally contributed to the conceptualization, writing, and editing of the manuscript.

CONFLICT OF INTEREST

The authors do not have any conflicts of interest.

REFERENCES

[1] Dahlman-Wright, K., et al., 2006. International union of pharmacology. LXIV. Estrogen receptors. Pharmacological Reviews 58(4):773–781.

[2] Adlanmerini, M., et al., 2014. Mutation of the palmitoylation site of estrogen receptor alpha in vivo reveals tissue-specific roles for membrane versus nuclear actions. Proceedings of the National Academy of Sciences of the United States of America 111(2):E283–E290.

[3] Maggi, A., 2011. Liganded and unliganded activation of estrogen receptor and hormone replacement therapies. Biochimica et Biophysica Acta 1812(8): 1054–1060.

[4] McKenna, N.J., et al., 2014. Nuclear receptor signaling: a home for nuclear receptor and coregulator signaling research. Nuclear Receptor Signaling 12: e006.

[5] Huang, W., et al., 2013. Cross-talk between the ligand- and DNA-binding domains of estrogen receptor. Proteins 81(11):1900–1909.

[6] Prossnitz, E.R., Barton, M., 2014. Estrogen biology: new insights into GPER function and clinical opportunities. Molecular and Cellular Endocrinology 389(1–2):71–83.

[7] Ciana, P., et al., 2001. Engineering of a mouse for the in vivo profiling of estrogen receptor activity. Molecular Endocrinology 15(7):1104–1113.

[8] Ciana, P., et al., 2003. In vivo imaging of transcriptionally active estrogen receptors. Nature Medicine 9(1):82–86.

[9] Della Torre, S., Maggi, A., 2017. Sex differences: a resultant of an evolutionary pressure? Cell Metabolism 25(3):499–505.

[10] Della Torre, S., et al., 2014. Energy metabolism and fertility: a balance preserved for female health. Nature reviews. Endocrinology 10(1):13–23.

[11] Lemmen, J.G., et al., 2004. Tissue- and time-dependent estrogen receptor activation in estrogen reporter mice. Journal of Molecular Endocrinology 32(3): 689–701.

[12] Roy, A.K., Chatterjee, B., 1983. Sexual dimorphism in the liver. Annual Review of Physiology 45:37–50.

[13] Holliday, R., 2006. Food, fertility and longevity. Biogerontology 7(3):139–141.

[14] Della Torre, S., et al., 2016. An essential role for liver ERalpha in coupling hepatic metabolism to the reproductive cycle. Cell Reports 15(2):360–371.

[15] Della Torre, S., et al., 2011. Amino acid-dependent activation of liver estrogen receptor alpha integrates metabolic and reproductive functions via IGF-1. Cell Metabolism 13(2):205–214.

[16] Ciana, P., et al., 2005. Estrogenic activities in rodent estrogen-free diets. Endocrinology 146(12):5144–5150.

[17] Baardman, M.E., et al., 2013. The role of maternal-fetal cholesterol transport in early fetal life: current insights. Biology of Reproduction 88(1):24.

[18] Woollett, L.A., 2011. Review: transport of maternal cholesterol to the fetal circulation. Placenta 32(Suppl. 2):S218–S221.

[19] Catalano, P.M., Hollenbeck, C., 1992. Energy requirements in pregnancy: a review. Obstetrical & Gynecological Survey 47(6):368–372.

[20] Montelongo, A., et al., 1992. Longitudinal study of plasma lipoproteins and hormones during pregnancy in normal and diabetic women. Diabetes 41(12): 1651–1659.

[21] Butte, N.F., 2000. Dieting and exercise in overweight, lactating women. New England Journal of Medicine 342(7):502–503.

[22] Kalhan, S., et al., 1997. Glucose turnover and gluconeogenesis in human pregnancy. The Journal of Clinical Investigation 100(7):1775–1781.

[23] Lim, S.S., et al., 2013. The effect of obesity on polycystic ovary syndrome: a systematic review and meta-analysis. Obesity Reviews: An Official Journal of the International Association for the Study of Obesity 14(2):95–109.

[24] Clark, J.M., et al., 2002. Nonalcoholic fatty liver disease. Gastroenterology 122(6):1649–1657.

[25] McKenzie, J., et al., 2006. Effects of HRT on liver enzyme levels in women with type 2 diabetes: a randomized placebo-controlled trial. Clinical Endocrinology 65(1):40–44.

[26] Wilfred de Alwis, N.M., Day, C.P., 2008. Genes and nonalcoholic fatty liver disease. Current Diabetes Reports 8(2):156–163.

[27] Villa, A., et al., 2012. Tetradian oscillation of estrogen receptor α is necessary to prevent liver lipid deposition. Proc Natl Acad Sci U S A 109(29): 11806–11811.

[28] Hart-Unger, S., et al., 2017. Hormone signaling and fatty liver in females: analysis of estrogen receptor α mutant mice. International Journal of Obesity 41(6):945–954.

[29] Gambarin-Gelwan, M., et al., 2007. Prevalence of nonalcoholic fatty liver disease in women with polycystic ovary syndrome. Clinical Gastroenterology

and Hepatology: The Official Clinical Practice Journal of the American Gastroenterological Association 5(4):496—501.

[30] Alpanes, M., et al., 2017. Combined oral contraceptives plus spironolactone compared with metformin in women with polycystic ovary syndrome: a one-year randomized clinical trial. European Journal of Endocrinology 177(5): 399—408.

[31] Elsheikh, M., et al., 2001. Hormone replacement therapy may improve hepatic function in women with Turner's syndrome. Clinical Endocrinology 55(2):227—231.

[32] Gravholt, C.H., 2004. Epidemiological, endocrine and metabolic features in Turner syndrome. European Journal of Endocrinology 151(6):657—687.

[33] Yang, X., et al., 2006. Tissue-specific expression and regulation of sexually dimorphic genes in mice. Genome Research 16(8):995—1004.

[34] Sugathan, A., Waxman, D.J., 2013. Genome-wide analysis of chromatin states reveals distinct mechanisms of sex-dependent gene regulation in male and female mouse liver. Molecular and Cellular Biology 33(18):3594—3610.

[35] Gonzalez, F.J., Lee, Y.H., 1996. Constitutive expression of hepatic cytochrome P450 genes. The FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology 10(10):1112—1117.

[36] Kushlan, M.C., et al., 1981. Sex differences in hepatic uptake of long chain fatty acids in single-pass perfused rat liver. The Journal of Lipid Research 22(3):431—436.

[37] Lorbek, G., et al., 2013. Sex differences in the hepatic cholesterol sensing mechanisms in mice. Molecules 18(9):11067—11085.

[38] Soler-Argilaga, C., et al., 1975. The effect of sex on the uptake of very low density lipoprotein triglyceride fatty acid from the plasma of the rat in vivo. Biochemical and Biophysical Research Communications 66(4):1237—1242.

[39] Hewitt, K.N., et al., 2004. Estrogen replacement reverses the hepatic steatosis phenotype in the male aromatase knockout mouse. Endocrinology 145(4): 1842—1848.

[40] Heine, P.A., et al., 2000. Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. Proc Natl Acad Sci U S A 97(23): 12729—12734.

[41] Matic, M., et al., 2013. Estrogen signalling and the metabolic syndrome: targeting the hepatic estrogen receptor alpha action. PLoS One 8(2):e57458.

[42] Qiu, S., et al., 2017. Hepatic estrogen receptor α is critical for regulation of gluconeogenesis and lipid metabolism in males. Scientific Reports 7(1): 1661—1668.

[43] MacLusky, N.J., Naftolin, F., 1981. Sexual differentiation of the central nervous system. Science 211(4488):1294—1302.

[44] Arnold, A.P., Gorski, R.A., 1984. Gonadal steroid induction of structural sex differences in the central nervous system. Annual Review of Neuroscience 7: 413—442.

[45] Harada, N., 1998. Localized aberrant expression of cytochrome P450 aromatase in primary and metastatic malignant tumors of human liver. Journal of Clinical Endocrinology & Metabolism 83(2):697—702.

[46] Agarwal, V.R., et al., 1998. Molecular basis of severe gynecomastia associated with aromatase expression in a fibrolamellar hepatocellular carcinoma. Journal of Clinical Endocrinology & Metabolism 83(5):1797—1800.

[47] Harada, N., et al., 1993. Tissue specific expression of the human aromatase cytochrome P-450 gene by alternative use of multiple exons 1 and promoters, and switching of tissue-specific exons 1 in carcinogenesis. Proceedings of the National Academy of Sciences of the United States of America 90(23):11312—11316.

[48] Toda, K., et al., 1994. Expression of the gene encoding aromatase cytochrome P450 (CYP19) in fetal tissues. Molecular Endocrinology 8(2):210—217.

[49] Yamamoto, T., et al., 1984. Estrogen biosynthesis in human liver — a comparison of aromatase activity for C-19 steroids in fetal liver, adult liver and hepatoma tissues of human subjects. Endocrinologia Japonica 31(3): 277—281.

[50] Gustafsson, J.A., et al., 1981. Role of the hypothalamo-pituitary-liver axis in sex differences in susceptibility of the liver to toxic agents. Environmental Health Perspectives 38:129—141.

[51] Bellinger, L.L., Williams, F.E., 1986. Liquid sucrose and fructose intake in male and female liver denervated rats. Brain Research Bulletin 17(6):835—846.

[52] Wiwi, C.A., Waxman, D.J., 2004. Role of hepatocyte nuclear factors in growth hormone-regulated, sexually dimorphic expression of liver cytochromes P450. Growth Factors 22(2):79—88.

[53] MacGeoch, C., et al., 1985. Hypothalamo-pituitary regulation of cytochrome P-450(15) beta apoprotein levels in rat liver. Endocrinology 117(5):2085—2092.

[54] Norstedt, G., Palmiter, R., 1984. Secretory rhythm of growth hormone regulates sexual differentiation of mouse liver. Cell 36(4):805—812.

[55] Jansson, J.O., et al., 1985. Imprinting of growth hormone secretion, body growth, and hepatic steroid metabolism by neonatal testosterone. Endocrinology 117(5):1881—1889.

[56] APA, 2013. Diagnostic and statistical manual of mental disorders, 5th ed. A.P. Association.

[57] WHO, 1993. The ICD-10 classification of mental and behavioural disorders : diagnostic criteria for research. G.W.H. Organization.

[58] Velho, I., et al., 2017. Effects of testosterone therapy on BMI, blood pressure, and laboratory profile of transgender men: a systematic review. Andrology 5(5):881—888.

[59] Jarin, J., et al., 2017. Cross-sex hormones and metabolic parameters in adolescents with gender dysphoria. Pediatrics 139(5).

