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To my family

ABSTRACT

Heli Jokela

Outcome of *Hsd17b1* gene deletion in ovarian and adipose tissue function: alterations resulting from both HSD17B1 enzyme inhibition and the off-target effect on the *Naglu* gene

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Steroids comprise a group of hormones that are, in the traditional view, secreted by the adrenal cortex, testes and ovaries, as well as the placenta during pregnancy and that reach their target organs via blood circulation. However, the concentration of available ligand for nuclear receptor binding is also regulated by target tissue metabolism. Hydroxysteroid (17beta) dehydrogenases (HSD17Bs) are a group of enzymes involved in this intracrine regulation of sex steroids. Among the HSD17Bs, HSD17B1 is an enzyme that catalyzes the reduction of estrone to estradiol and the reduction of androstenedione to testosterone. To elucidate the physiological function of HSD17B1, we generated a knockout mouse model with a disrupted *Hsd17b1* gene, in which the entire coding region of *Hsd17b1* was replaced with a LacZ/Neo insertion. Pubertal onset and estrous cycle were found to be normal in the homozygous Hsd17b1-LacZ/Neo females. However, the mice were subfertile and had defects in pregnancy maintenance, likely affected by an imbalance in ovarian steroid synthesis. In addition, the Hsd17b1-LacZ/Neo males presented with reduced fat mass, increased lean mass, and fatty liver, and the mice had an improved tolerance to high-fat diet-induced obesity. Surprisingly, the deletion of the *Hsd17b1* gene disrupted the expression of an upstream neighboring gene, N-acetyl-alpha-glucosaminidase (*Naglu*). Furthermore, biochemically and morphologically similar metabolic phenotypes were observed in the *Naglu* knockout mice, while their fertility was normal. This observation together with observations from other analyses, indicated that the metabolic dysfunctions in the Hsd17b1-LacZ/Neo males were related to the downregulation of *Naglu* expression and not to the inhibition of HSD17B1 activity. In summary, this study elucidates the importance of HSD17B1 enzyme in female fertility and provides an example of how the altered genomic structure can affect the functions of neighboring genes, leading to severe off-target effects.

Keywords: Hsd17b1, knockout, fertility, adiposity, gene targeting, Naglu

TIIVISTELMÄ

Heli Jokela

Hsd17b1-geenin poiston seuraukset munasarjojen ja rasvakudoksen toimintaan: HSD17B1-entsyymin inhibition ja naapurigeeni *Naglu*:n häiriintyneen toiminnan aiheuttamat muutokset

Turun yliopisto, Lääketieteellinen tiedekunta, Biolääketieteen laitos, Fysiologia, Lääketutkimuksen tohtoriohjelma, Turku

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Sukupuolihormonit tuotetaan perinteisen näkemyksen mukaan lisämunuaisissa, munasarjoissa, kiveksissä ja raskausaikana istukassa, joista hormonit tavoittavat kohdekudoksensa verenkierron kautta. Nykytiedon mukaan sukupuolihormonien pitoisuuksia säädellään ja niiden rakennetta muokataan entsymaattisesti myös kohdekudoksessa, ja näin paikallinen aineenvaihduntakin säätelee hormonivaikutuksia. Hydroksisteroidi(17-beeta)dehydrogenaasit (HSD17B:t) ovat ryhmä entsyymejä, jotka osallistuvat kohdekudoksissa tapahtuvaan sukupuolihormonien säätelyyn. HSD17B1-entsyymin on osoitettu *in vitro* katalysoivan heikon estrogeenin estronin muuttumista voimakkaasti vaikuttavaksi estradioliksi ja heikon androgeenin androstenedionin aktivoitumista voimakkaasti vaikuttavaksi testosteroniksi. Selvittääksemme tarkemmin HSD17B1-entsyymin fysiologiset toiminnot ja merkityksen teimme poistogeeniset hiiret (Hsd17b1-LacZ/Neo-hiiret), joissa koko *Hsd17b1*-geenin koodaava alue korvattiin raportoijageeniä (*lacZ*) ilmentävällä DNA-fragmentilla. Murrosiän puhkeamisen ja estruskierron todettiin olevan normaaleja Hsd17b1-LacZ/Neo-naarailla, mutta muuntuneen steroidisynteesin vuoksi hiiret olivat subfertiilejä johtuen ongelmista raskauden alkamisessa. Lisäksi Hsd17b1-LacZ/Neo-uroksilla oli vähentynyt rasvamassa, lisääntynyt lihasmassa, rasvamaksa ja toleranssi rasvaisen ruokavalion aiheuttamalle lihavuudelle. Yllättäen havaitsimme *Hsd17b1*-geenin poiston häiritsevän merkittävästi viereisen geenin N-asetyyli-alfa-glukosaminidaasin (*Naglu*) ilmentymistä. *Naglu*-poistogeenisten hiirten metaboliailmiasu osoittautui biokemiallisesti ja morfologisesti samanlaiseksi kuin *Hsd17b1*-poistogeenisillä hiirillä. Näin ollen *Hsd17b1*-poistogeenisissä hiirissä havaitut rasva-aineenvaihdunnan muutokset johtuivat alentuneesta *Naglu*-geenin ilmentymisestä eivätkä liittyneet HSD17B1-entsyymin puutokseen. Toisin kuin Hsd17b1-LacZ/Neo-naarailla *Naglu*-poistogeenisten naaraiden lisääntymisbiologia oli normaali. Tässä tutkimuksessa osoitettiin HSD17B1-entsyymin välttämättömyys naaraiden hedelmällisyydelle. Lisäksi työ osoitti, että geenilokuksen DNA-jakson muuntaminen voi merkittävästi vaikuttaa naapurigeenien ilmentymiseen.

Avainsanat: Hsd17b1, lisääntyminen, rasva-aineenvaihdunta, geeninpoisto, Naglu

TABLE OF CONTENTS

ABBREVIATIONS

Acaca	acetyl-CoA carboxylase alpha
Actb	actin beta
5α-Adione	5α-Androstenedione
20alpha-HSD	20alpha-hydroxysteroid dehydrogenase
Adione	androstenedione
ALK4	activin receptor-like kinase 4
AKR	aldoketoreductase
BAT	brown adipose tissue
bp	base pairs
cAMP	cyclic adenosine monophosphate
Cas9	CRISPR associated protein 9
CDM	chaperone-mediated autophagy
CL	corpus luteum / corpora lutea
CLEAR	coordinated lysosomal expression and regulation element
Coasy	coenzyme A synthase
CRISPR	clustered regularly-interspaced short palindromic repeats
CYP11A1	cholesterol side-chain cleavage enzyme (P450scc)
CYP17A1	steroid 17-alpha-hydroxylase/17,20 lyase
CYP19A1	aromatase
Cyp51	cytochrome P450, family 51
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
Dhcr7	7-dehydrocholesterol reductase
Dhcr24	24-dehydrocholesterol reductase
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
dpc	days post coitum
E1	estrone
E2	estradiol
E3	estriol
EE	energy expenditure
ES	embryonic stem cell
FAS	fatty acid synthesis
Fasn	fatty acid synthase
FSH	follicle-stimulating hormone
GAG	glycosaminoglycan
GlcNAC	N-Acetylglucosamine

GM	genetically modified
GnRH	gonadotropin-releasing hormone
gRNA	guide ribonucleic acid for CRISPR/Cas9
gWAT	gonadal white adipose tissue
hATCB promoter	human b-actin promoter
HE	hematoxylin-eosin
HEK 293	human embryonic kidney cells 293
HEZ	heterozygous
HDR	homology directed repair
HFD	high-fat diet
Hmgcr	3-hydroxy-3-methylglutaryl-coenzyme A reductase
HOZ	homozygous
HS	heparan sulfate
HsCBR4	carbonyl reductase type 4
HSD17B	hydroxysteroid (17beta) dehydrogenase
HSD17B1	human hydroxysteroid (17beta) dehydrogenase 1 gene
HSD17B1P1	human hydroxysteroid (17beta) dehydrogenase 1 pseudogene
Hsd17b1-LacZ/Neo	Hsd17b1 knockout mouse with LacZ/Neo insertion
Hsd17b1-LacZ	Hsd17b1 knockout mouse with LacZ insertion
HSD17B1TG	transgenic mouse expressing human HSD17B1
HSKAR	β-ketoacyl thioester reductase
hUBC promoter	human ubiquitin C promoter
KAR	3-ketoacyl-CoA reductase
L19	ribosomal gene 19
lacZ	beta-D-galactosidase
LAMP1	lysosomal-associated membrane protein 1
LIMP2	lysome membrane protein 2
LD	lipid droplet
LH	luteinizing hormone
Lhcgr	luteinizing hormone/choriogonadotropin receptor
LTR	long terminal repeat
miRNA	micro ribonucleic acid
mRNA	messenger ribonucleic acid
MPS	mucopolysaccharidosis
NAD(P)(H)	nicotinamide adenine dinucleotide (phosphate)
NAFLD	non-alcoholic fatty liver disease
Naglu	N-acetyl-alpha-glucosaminidase
Naglu-Neo	Naglu knockout mouse with Neo insertion
NASH	nonalcoholic steatohepatitis

ND	normal diet/chow
NEFA	non-esterified fatty acid
neo	neomysin
NHEJ	non-homologous end joining
Nsdhl	NAD(P) dependent steroid dehydrogenase-like
P4	progesterone
PAS	Periodic acid–Schiff staining
pck	polycystic kidney
Pgk promoter	phosphoglycerate kinase promoter
PRAP	prolactin receptor associated protein
qRT-PCR	quantitative real time polymerase chain reaction
RER	respiratory exchange ratio
RNA	ribonucleic acid
RT	room temperature
SA	splice acceptor
SCDR9	short-chain dehydrogenase/reductase 9
SCHAD	L-3-hydroxyacyl-CoA dehydrogenase
scWAT	subcutaneous white adipose tissue
SDR	short dehydrogenase reductase
SINE	short interspersed nuclear element
SMAD2	SMAD Family Member 2
T	testosterone
TAG	triacylglycerol/triglyceride
TATA box	eukaryotic DNA sequence, usually TATAAATA
TFEB	transcription factor EB
TG	transgenic
TGF-beta	transforming growth factor beta
UTR	untranslated region
WAT	white adipose tissue
WT	wild type

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original publications, which are referred to in the text by Roman numerals I-II:

I. Hakkarainen J, **Jokela H**, Pakarinen P, Heikelä H, Kätkänaho L, Vandenput L, Ohlsson C, Zhang F, Poutanen M (2015) "Hydroxysteroid (17beta) dehydrogenase 1 deficient female mice present with normal puberty onset but severe subfertility due to a defect in luteinization and progesterone production" FASEB J 29: 3806-3816

II. **Jokela H**, Hakkarainen J, Kätkänaho L, Pakarinen P, Ruohonen S, Tena-Sempere M, Zhang F, Poutanen M (2017) "Deleting the mouse Hsd17b1 gene results in a hypomorphic Naglu allele and a phenotype mimicking a lysosomal storage disease" Sci Rep 27:16406

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1 INTRODUCTION

Sex steroids, namely estradiol (E2) and testosterone (T), are crucial in female and male reproduction. Sex steroids induce the development and growth of reproductive organs and are thus essential for puberty and fertility. However, myriad of other endocrine actions are also controlled by sex steroid hormones, including glucose control, insulin sensitivity, lipid metabolism and, finally, obesity. Biologically, the most effective sex steroids are the 17beta-hydroxy forms (E2, T), which are produced by the gonads. However, it has become increasingly evident that both estrogens and androgens are synthetized also locally in peripheral tissues such as breast, prostate and adipose tissue. Sex steroid conversion is influenced by the presence of different steroidogenic and metabolizing enzymes. Among these enzymes, hydroxysteroid (17beta) dehydrogenases (HSD17Bs) act in the final steps of sex steroid biosynthesis, converting 17keto-steroids, estrone (E1) and androstenedione (Adione) to more potent 17beta-hydroxysteroids (E2 and T), and *vice versa*. At present, 14 different HSD17B enzymes have been characterized with broad expression profiles, and the data have indicated the importance of these enzymes in the activation and inactivation of steroids both in gonads and in peripheral tissues. Thus, HSD17Bs are important factors in the regulation of sex steroid actions. In addition, HSD17Bs have been demonstrated to be involved in the pathogenesis of hormone-dependent diseases, such as breast cancer and endometriosis, and consequently, HSD17Bs are considered to be potential drug targets. HSD17B1 is an enzyme that predominantly converts E1 to E2, but HSD17B1 has also been shown to catalyse the conversion of Adione to T. The gene coding for HSD17B1 is especially expressed in the granulosa cells of both the human and rodent ovary and in the human placenta. Interestingly, its expression has also been detected in several sex steroid target tissues, including adipose tissue and the testis, indicating a local steroidogenic function for the HSD17B1 enzyme.

Mouse models are typically used as tools for studying reproduction and metabolic systems, and due to complex organ system interactions, these endocrine processes are difficult to study *in vitro*. In full knockout mouse models, a gene of interest can be partly or fully deleted to disrupt the coding of a protein and thus provide knowledge of the *in vivo* function of the protein. Unfortunately, genomic modification can cause unwanted off-target effects on the neighbouring genes, which can complicate the conclusions formed from the observed phenotype.

In this thesis we set out to study how the deletion of the *Hsd17b1* mouse gene affected ovarian and adipose tissue functions to obtain information on the role of the HSD17B1 enzyme in the regulation of endocrine functions. The association

between HSD17B1 and estrogen biosynthesis makes the enzyme an attractive target for studies of both normal physiological conditions and pathophysiological conditions. Therefore, a mouse model with the whole coding region of *Hsd17b1* deleted was generated. HSD17B1 activity was revealed to be essential to the maintenance of pregnancy, as the lack of HSD17B1 activity resulted in severe subfertility in the female mice. Interestingly, we also found that deletion of the *Hsd17b1* gene had an effect on adiposity and glucose metabolism in the mice. However, the metabolic phenotype was discovered to be a result of the downregulation of N-acetyl-alpha-glucosaminidase (*Naglu*), a gene that is located close to *Hsd17b1* in the genome.

2 REVIEW OF LITERATURE

2.1 Gene targeting

A variety of strategies have been employed to generate genetically modified (GM) animals to understand how the gene of interest functions *in vivo*. Mice are inexpensive and easy to maintain, which is why mice are commonly used for this purpose. In addition, the mouse genome has been characterized and the majority of genes operate similarly in mice and humans (Behringer et al., 2014). Practically, mice have similar internal organs and a similar physiology to humans, which typically permits translation of the results obtained from mice to humans. The mouse genome can be modified in many different ways. Overexpression can be created by inserting a copy (or multiple copies) of a gene, and gene deletion by DNA replacement is a common method for creating knockout mouse models. These genetically modified models may reveal mechanistic pathways that underline specific outcome or diseases.

2.1.1 Methods for generating genetically modified animals

2.1.1.1 Pronuclear injection to generate transgenic mice

For creating transgenic mice, foreign DNA is typically presented into the mouse genome by two methods. DNA can be delivered to mouse embryos at different developmental stages by retroviral infection. However, this method is not in routine use because of technical problems, *e.g.* retroviral-mediated approach can cause multiple chromosomal integration of the transgene (Behringer et al., 2014). Therefore, pronuclear injection is more commonly used for generating transgenic mice. To express a transgene in mice, the construct, including the full coding sequence of the gene of interest and a promoter that determine the expression strength and specificity, are directly microinjected into the pronuclei of fertilized one-cell mouse embryos. The site of integration of injected DNA is random as is the number of copies (Palmiter and Brinster, 1986). The embryos are transferred into the oviducts of pseudopregnant female mice, which consequently produce the founders carrying the transgene with different frequencies (Figure 1).

2.1.1.2 Generating knockout mice by homologous recombination

While pronuclear injection produces a random modification in a genome, gene targeting provides a site-specific genome alteration. With a homologous recombination based method, one can inactivate the targeted gene (gene knockout), or the targeted gene can be replaced by an alternative active gene (gene knock-in). Knockout mice have traditionally been produced by homologous recombination, where the replacement vector contains two flanking regions of DNA that are homologous to the genomic target locus. For the exchange of nucleotide sequences between the targeting construct and genomic DNA, high sequence similarity or an identical DNA sequence is essential (Behringer et al., 2014). The gene targeting method to generate mice with altered genomes utilizes mouse embryonic stem (ES) cells in which the artificial genetic material is first inserted. When this method was introduced in late 1980's (Smithies et al., 1985), the efficiency of the method was driven by two main factors. First, gene modification could be performed *in vitro* with ES cells rather than in a whole organism. Second, the selection of targeted cells straightforwardly occurred in two steps:

I. The ES cells, containing the incorporated targeting vector, were identified by their antibiotic resistance due to the engineering of a neomycin resistance cassette into the targeting vector (Cheah and Behringer, 2000), and

II. The ES cells, with homologous recombination in the targeted locus, were identified by including a thymidine kinase cassette downstream of the homology region of the construct (Cheah and Behringer, 2000; Mansour et al., 1988).

Thereafter, the targeted ES cells are injected into the cavity of the blastocysts (embryonic day (E) 3.5), and then, the chimeric embryos are transferred into the uterine cavity of pseudopregnant females. A variable number of chimeric mice are subsequently born, and the presented genetic modifications is passed on to offspring by breeding chimeric mice with wild type (WT) mice, if the genetically modified ES cells have contributed to the germ cells (Figure 1, Behringer *et al.*, 2014).

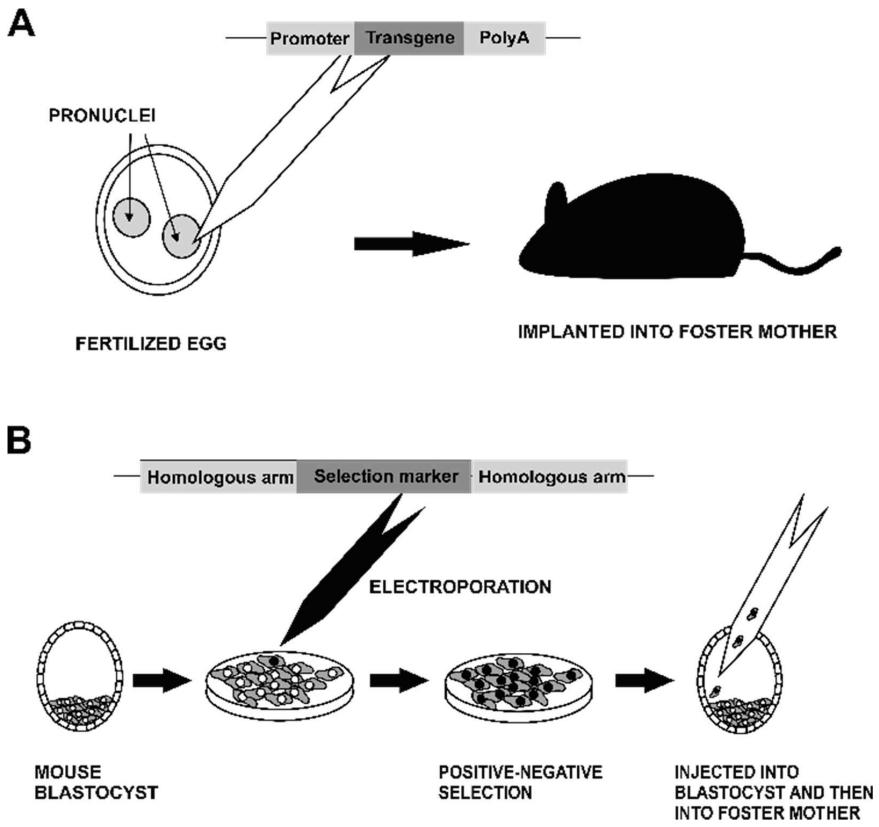

Figure 1. Gene manipulation by pronuclear injection and by homologous recombination. A) For the pronuclear injection, the DNA construct is injected into the pronuclei of a single-cell-stage fertilized egg. The injected eggs are then transferred into the oviducts of pseudopregnant foster mice. B) To create the knockout mice, the knockout ES cell line is generated by homologous recombination. These targeted ES cells are injected into the blastocysts of donor mice, and the injected blastocysts are then transferred into the uteri of a pseudopregnant foster mice.

To facilitate the generation of GM mice with conditional gene ablation/modification a Cre/loxP recombination-based method is currently very popular. With this method, the DNA sequence (*e.g.* selection marker or gene of interest) can be deleted by flanking the sequence with loxP sites that are 34 base-pairs (bp)-long DNA sequences recognized by bacterial Cre recombinase. These loxP sites are first introduced into the mouse genome as discussed above. Second, the floxed mice are bred with GM mice, in which Cre recombinase is expressed in specific tissues or in a specific cell type. Cre can also be transiently expressed during the developmental stages of tissues or cells. In addition, if fused with a tamoxifen-inducible estrogen receptor mutant the Cre activity can be induced at the preferred location and time. This method enables functional studies of the gene of interest in specific

tissues or developmental stages rather than in the whole organism (Behringer et al., 2014; Bouabe and Okkenhaug, 2013; Hadjantonakis et al., 2008).

Another method for generating GM mice, VelociGene, is an automated high-throughput process that uses bacterial artificial chromosome (BAC)-based targeting vectors to modify many genes in parallel (Valenzuela et al., 2003). With the VelociGene method, it is possible to generate mice with genetic alteration, including conditional alleles, point mutations or gene switches, in genes of interest. The approach provides nucleotide precision, but the method can also delete large genomic regions. The introduction of positive and negative markers is not necessary for this method. In addition, VelociGene has been used to generate a vector, which deletes the whole coding region from the ATG of the targeted gene to the 3' UTR as efficiently as smaller deletions (Valenzuela et al., 2003).

2.1.1.3 Gene trapping to create mutant mice

The genome can be also modified by gene trapping, which is a random mutation method used in a high-throughput mode (Abuin et al., 2007; Gossler et al., 1988; Guan et al., 2010). Although this technique is not as specific as homologous recombination, trapping can be used to knock out several mouse genes in a short period of time. Electroporation or retroviral infection can be used to introduce three trap vector types into the genome: the enhancer-, promoter- and gene-trap vectors (Allen et al., 1988; Friedrich and Soriano, 1991; Gossler et al., 1988; Reddy et al., 1991). Trapping vectors commonly have an element that includes a selection marker (a combination of *β-galactosidase* and *neomycin*-resistance genes) lined with a splice acceptor (SA) and a polyA signal. The vector insertions into the mouse genome occur randomly in the ES cells, and the locations of the inserts are determined afterwards. When the insertion is located in an intronic position of a gene (favorable in the first intron), fusion transcripts are formed during gene expression. These include exons upstream of the targeted intron, the reporter gene and a selection marker, which are fused in the newly formed transcript via a novel SA of the gene-trap vector. As a result, only a truncated protein from the endogenous gene is formed (Stanford et al., 2001).

2.1.1.4 CRISPR/Cas9

A more recent approach for generating genetically modified animal models is the CRISPR/Cas9 (clustered regularly interspaced short palindromic repeat/CRISPR-associated protein 9) technique. The principal mechanism of the CRISPR/Cas9

method is that both the Cas9 RNA and a "guide" RNA (gRNA) sequence are injected into a cell. Furthermore, the gRNA targets the gene of interest via the homologous sequence and recruits Cas9 to the corresponding DNA sequence on the targeted genome. Then, the Cas9 nuclease cuts both DNA strands, which can be repaired with two different approaches resulting in different types of genomic disruptions. First, the use of non-homologous end joining (NHEJ) results in a permanent disruption of the target sequence. On the other hand, exact mutations and knock-ins can be generated with a homology-directed repair (HDR) template (Jinek et al., 2012; Sander and Joung, 2014; Tschaharganeh et al., 2016).

2.1.2 Off-target effects in GM mice

Over the decades, a great deal of knowledge has been gained through studies with genetically modified animals. Unfortunately, modification of the genomic sequence can have unintended consequences on the regulation of adjacent or distant transcriptional units. The most widely used technique to date for disrupting the gene function in mice is based on replacing the whole gene of interest or a part of the gene with a cassette that includes DNA fragments coding for a bacterial beta-galactosidase reporter (*lacZ*) and/or a neomycin (*neo*) resistance genes. Besides successfully eliminating the target gene, insertion of approximately 5 kb long cassette can alter gene regulation of nearby genes (Pan et al., 2016; Zuniga et al., 2004). These off-target effects can interfere with the interpretation of the observed phenotype and lead to wrong conclusions about the function of the eliminated gene of interest.

Some of the effects of gene targeting on the neighboring genes can be caused by co-regulation of gene clusters. When chromatin opens for the purpose of gene expression, the opening might promote the expression of other genes in the area of the open region; gene regions are usually up- or downregulated together (Gierman et al., 2007; Sproul et al., 2005). There are also many intragenic elements identified within introns and in 5' and 3' untranslated regions (UTRs, Kleinjan and van Heyningen, 2005; Barrett, Fletcher and Wilton, 2012). These include promoters, repressors, enhancers and cis-acting elements, and of these intragenic elements, enhancers and repressors can influence even distant promoters through chromatin looping (Holwerda and de Laat, 2012; Nolis et al., 2009). When a part of the genome is deleted, the deletion may in parallel also delete a promoter or enhancer region for a flanking gene. Transcriptional enhancers are short DNA segments that are able to radically increase transcription from the promoter of a target gene. Enhancers do not act on the promoters itself, but they are bound to the promoter re-

gion by activator proteins. Enhancers can exert their effect over short or long distances either from upstream, downstream, or from within a transcription unit. The distance can be up to 1 Mbp, but still enhancers regulate only specific genes (Zabidi and Stark, 2016). Studies in humans and mice have shown that promoter regions are intrinsically bidirectional and regulate the surrounding genes accordingly (Jin et al., 2017; Koyanagi et al., 2005; Lin et al., 2007). Consequently, removal of a part of the genome may cause local effects because of the loss of a bidirectional promoter (West et al., 2016). For example, *hUBC* promoter has been shown to have some bidirectional regulatory effects (Chen et al., 2011). In addition, the genomic locus of interest can contain sequences coding for miRNAs, which affect the transcript stability (Lettice et al., 2002; Narboux-Nême et al., 2012; Zuniga et al., 2004). The unintentional disruption of miRNA sequences is especially likely when the whole gene is deleted since many of the sequences coding for miRNAs are present within the introns of protein coding genes (Rodriguez, 2004). As miRNAs act in RNA silencing and post-transcriptional gene regulation, gene targeting may disrupt these processes and consequently affect the expression of other genes (Osokine et al., 2008).

The selection cassette inserted into the mouse genome during gene targeting can also have an influence on nearby genes (Meier et al., 2010; Olson et al., 1996; Pham et al., 1996; Ramirez-Solis et al., 1993; Scacheri et al., 2001; West et al., 2016). Whether the off-target effect on the neighboring genes is caused by the *neo* selection cassette sequence, including the promoter, or by targeting-induced disruption of the cis-acting regulatory elements is often difficult to determine. It has been demonstrated that, when the exogenous *Pgk* promoter is inserted as part of the *neo* selection cassette, embryonic lethality occurs already in heterozygous mice. During the targeting, the *neo* cassette is inserted in the opposite direction of the transcription of the targeted gene, causing the expression of a deviant transcript from the antisense strand driven by the *Pgk* promoter (Scacheri et al., 2001). There are also examples showing that different targeting strategies for the same gene result in different phenotypes (Olson et al., 1996; Ramirez-Solis et al., 1993). The insertion site and the size of the deletion can cause phenotypes ranging from complete viability of the homozygous mice to embryonic lethality (Olson et al., 1996). One explanation for the differences in the expression of the neighboring genes could be the stability and strength of the promoter used and the content of the selection cassette itself. Although *neo* is the most commonly used selection marker, the targeting vectors may have different promoters (*Pgk*, *hUBC* and *hATCB*) and the polyA signals (SV40 and polyA of *mPgk*), differentially affecting the expression of nearby genes (West et al., 2016).

2.2 HSD17B enzymes

Balanced sex steroid hormone metabolism is necessary for physiological functions, such as puberty and reproduction, in both humans and mice. The traditional understanding is that ovaries and testes act as sources of circulating sex steroids and that sex steroids reach their target tissues and cells through blood circulation. However, the circulating sex steroid concentrations do not necessarily correlate with the tissue concentrations (Huhtinen et al., 2012, 2014). A more comprehensive elucidation indicates that sex steroids are regulated by steroidogenic enzymes in neighboring cells and/or in target cells themselves. Thus, these paracrine and intracrine actions regulate the intratissue hormone levels (Labrie, 2015; Labrie and Belanger, 1988). Hydroxysteroid (17beta) dehydrogenase enzymes (HSD17Bs) are a family of local steroid metabolism regulators that catalyze the conversion of low activity 17-keto steroids to highly active 17beta-hydroxy steroids in the last steps of steroid hormone biosynthesis. The reaction occurs on position C17 of the carbon chain of the steroid structure, and the reaction is cofactor dependent (nicotinamide-adenine dinucleotide phosphate [NADP] and NADPH; Moeller and Adamski, 2009). The reactions catalyzed by HSD17Bs include the conversion of estrone (E1) to estradiol (E2), androstenedione (Adione) to testosterone (T), and 5α-androstenedione (5α-Adione) to dihydrotestosterone (DHT, Figure 2). In addition to having HSD17B activity, certain HSD17B enzymes have activity in other metabolic pathways, including the synthesis of cholesterol and lipids (Jokela et al., 2010; Rantakari et al., 2010).

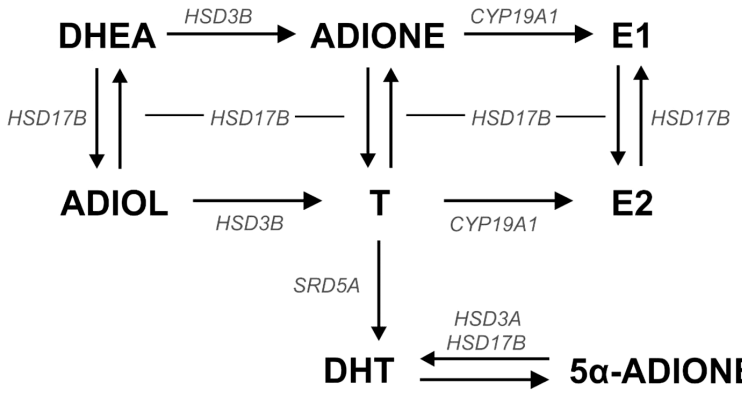

Figure 2. Final reactions on steroid biosynthesis pathway. Enzymes are shown near the arrows indicating chemical reactions. DHEA = dehydroepiandrosterone, ADIOL = androstenediol.

The HSD17B enzymes are numbered chronologically in order of identification, and to date 14 different HSD17Bs have been identified in vertebrates, of which 12

have been identified in humans. Furthermore, HSD1Bs 1-4 and HSD17Bs 6-14 belong to the short-chain dehydrogenase/reductase (SDR) family, and HSD17B5 is an aldo-keto reductase (AKR) (Moeller and Adamski, 2006, 2009; Peltoketo et al., 1999). SDRs comprise a large family of NADPH-dependent oxidoreductases that have only a few common sequence motifs and display similar mechanisms. The SDR enzymes have crucial functions in steroid, lipid, carbohydrate, amino acid and cofactor metabolism (Wu et al., 2007). The nomenclature for HSD17Bs is based on the genetic identity of the enzymes and on their functionality. However, tissue distribution, sequence homology, subcellular localization and substrate specificity vary considerably between the different HSD17B enzymes (Lin et al., 2013; Lukacik et al., 2006; Mindnich and Adamski, 2009; Moeller and Adamski, 2006, 2009; Peltoketo et al., 1999; Wu et al., 2007).

Sex steroids are involved in the pathogenesis of several hormone-dependent diseases, such as breast and prostate cancer. Consequently, altered expression of many of the HSD17B enzymes has been identified in various sex steroid target tissues in pathophysiological conditions (Haynes et al., 2010; Jansson et al., 2006; Stanbrough et al., 2006; Vihko et al., 2005, 2006). Thus, the HSD17B enzymes are considered to be potential drug targets for diseases, such as breast and prostate cancer, and inhibitors for several HSD17Bs, including HSD17B1,s are being developed (Beck et al., 2017; Laplante et al., 2009; Loriot et al., 2014; Marchais-Oberwinkler et al., 2011; Poirier, 2011; Wang et al., 2015).

2.2.1 Activity of the HSD17B enzymes in non-steroidal pathways

2.2.1.1 HSD17B2

HSD17B2 is an oxidative enzyme and is capable of converting E2, testosterone and DHT to their less active forms, namely E1, Adione and 5α-Adione, respectively. Additionally, the enzyme has 20alpha-HSD activity and thus converts 20α-hydroxy-progesterone to progesterone (Labrie et al., 1995; Wu et al., 1993). The expression of HSD17B2 is widespread in both human and mouse sex-steroid targeted tissues, with an especially high expression in the placenta and endometrium (Casey et al., 1994; Mustonen et al., 1997, 1998a). However, further studies with mice and humans have shown that the enzyme is also expressed throughout the gastrointestinal tract and in the liver and lungs (Mustonen et al., 1998b; Sano et al., 2001). The HSD17B2 enzyme has also been localized in the blood vessels of human abdominal adipose tissues (Fouad Mansour et al., 2015) and in enterocytes

as lipid droplet protein (Beilstein et al., 2013). A study with enterocytes has revealed that HSD17B2 interferes with triacylglycerol (TAG) secretion (Beilstein et al., 2013). The studies based on genetically modified mouse models suggest that in addition to functions in steroid hormone metabolism, HSD17B2 has a direct or indirect role in the retinoid metabolism (Rantakari et al., 2008; Zhongyi et al., 2007).

2.2.1.2 HSD17B4

HSD17B4 was first identified based on its ability to catalyze the oxidative reaction of E2 to E1 (Adamski et al., 1992). The enzyme has also been found to be widely expressed outside steroid target tissues, and the expression of human *HSD17B4* is quite ubiquitous with the highest mRNA expression levels in the liver, followed by the heart, prostate and testis (Adamski et al., 1996). Mouse *Hsd17b4* is expression is highest in the liver, kidney and skeletal muscle, and moderate expression has been detected in the heart, testis and lung (Normand et al., 1995). At the subcellular level, HSD17B4 is located in the peroxisome, which is an unlikely location for steroid-converting enzymes (Adamski et al., 1996; Markus et al., 1995). As the tissue distribution expression and cellular localization indicate, HSD17B4 is also involved in peroxisomal β-oxidation and trimming of very long chain fatty acids and bile acid degradation (Baes et al., 2000; Dieuaide-Noubhani et al., 1996, 1997; Huyghe et al., 2006).

2.2.1.3 HSD17B6

The human and rat HSD17B6 enzymes were first defined as retinol dehydrogenases (RoDHs) that have activity towards all-trans-retinol (Biswas and Russell, 1997). Human *HSD17B6* is mainly expressed in the liver and prostate (Bauman et al., 2006; Knuuttila et al., 2014; Liu et al., 2007), while the expression of mouse *Hsd17b6* is highly restricted to the liver (Su et al., 1999). Additionally, HSD3A, HSD3B and HSD17B activities, which are involved in androgen metabolism, have been described for the HSD17B6 enzyme (Biswas and Russell, 1997; Huang and Luu-The, 2000). Furthermore, HSD17B6 has been demonstrated to possess epimerase activity by functioning as a 3(α→β) hydroxysteroid epimerase (HSE), as HSD17B6 enzyme catalyzes both the reductive and oxidative reactions of androgens (Huang and Luu-The, 2000).

2.2.1.4 HSD17B7

The cDNA coding region of the hydroxysteroid (17beta) dehydrogenase 7 (HSD17B7) enzyme was first cloned from the rat corpus luteum and originally was identified as a prolactin receptor-associated protein (PRAP) (Duan et al., 1996). However, the functional role of PRAP protein with the prolactin receptor has not been studied further. Later, the corresponding mouse homolog for PRAP was found to have HSD17B activity, catalyzing the conversion of E1 to E2, and the protein was named HSD17B7 (Nokelainen et al., 1998). The recombinant human HSD17B7 enzyme converts DHT to 5α-androstane-3β and 17β-diol (3β-Adiol) with the same catalytic efficiency as measured for the E1 to E2 reaction, while mouse HSD17B7 converts DHT to both 3α-Adiol and 3β-Adiol with low efficiency (Törn et al., 2003). Furthermore, because amino acid sequence similarity between human HSD17B7 and the yeast 3-keto sterol reductase (*Saccharomyces cerevisiae*) ERG27 protein was evident, HSD17B7 was predicted to possess a 3-ketosteroid reductase activity (Breitling et al., 2001). Consequently, human HSD17B7 has been demonstrated *in vitro* to catalyze the zymosterone to zymosterol reaction, which is a reaction that is essential for postsqualene cholesterol biosynthesis (Marijanovic et al., 2003). The supposed role of HSD17B7 in cholesterol biosynthesis gained further support when a similar expression pattern for the enzyme, along with other cholesterogenic enzymes, was observed during mouse embryonic development (Laubner et al., 2003; Marijanovic et al., 2003). We also generated a knockout mouse line by disrupting the *Hsd17b7* gene by inserting a *neo* cassette into exon 2, and our results conclusively demonstrated the role of HSD17B7 in cholesterol biosynthesis (Jokela et al., 2010). The homozygous knockout mice died *in utero* on embryonic day (E) 10.5 due to the heart developmental and vasculature defects in the embryo and due to disrupted yolk sac structures. In addition, the mutant embryos presented with holoprosencephaly, a syndrome associated with a defective cholesterol biosynthesis (Haas and Muenke, 2010). Our data demonstrated marked alterations in the cholesterol biosynthesis intermediate concentrations in a manner expected with the measured cholesterogenic activity for HSD17B7 *in vitro*. Upstream intermediates, including lanosterol and squalene, accumulated in the *Hsd17b7* knockout (HSD17B7KO) mouse embryos, while the levels of cholestenol, lathosterol and desmosterol, which represented the later steps of cholesterol biosynthesis, were markedly reduced. Interestingly the total cholesterol concentrations were not altered in the embryos, indicating maternal contribution through the placenta. Thus, our data, together with the analysis by Shehu *et al*. (Shehu et al., 2008), unquestionably showed the essential function of HSD17B7 *in vivo* in the cholesterol biosynthesis of mice (Jokela et al., 2010).

2.2.1.5 HSD17B8

HSD17B8 was first identified as Ke6 in mice with polycystic kidney (pck) disease, (Aziz et al., 1993) and the expression of *Ke6* was downregulated in all pck mouse models (Maxwell et al., 1995). It has been later shown that Ke6 is an HSD17B enzyme and has the capacity to convert estradiol to estrone (Fomitcheva et al., 1998). The expression of the HSD17B8 enzyme has been predominantly detected in the kidney, liver (Aziz et al., 1993), testis, ovary (Fomitcheva et al., 1998), and several other tissues (Pelletier et al., 2005). More recently, human HSD17B8 was shown to physically interact and form a functional β-ketoacyl thioester reductase (HsKAR) $\alpha_2\beta_2$-tetramer with human carbonyl reductase type 4 (HsCBR4). The interaction indicates a role for this subunit in the mitochondrial fatty acid synthesis (FAS) pathway (Chen et al., 2009; Venkatesan et al., 2014).

2.2.1.6 HSD17B10

HSD17B10 was first discovered as a short-chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD) and has been shown to catalyze the oxidation of branched and straight fatty acids (He et al., 1998). The enzyme is localized to mitochondria as a homotetrameric protein (He et al., 1998). HSD17B10 is a multifunctional enzyme. HSD17B10 takes part in the oxidation of branched-chain fatty acids, isoleucine and xenobiotics, and HSD17B10 participates in the metabolism of neuroactive steroids and sex steroids (He and Yang, 2006; He et al., 1999, 1998, Yang et al., 2009, 2007). HSD17B10 plays a role in neurodegenerative disorders. For example, elevated levels of HSD17B10 are present in Alzheimer's disease (Yang et al., 2014).

2.2.1.7 HSD17B12

HSD17B12 was first shown to be involved in a metabolic pathway other than steroid metabolism. Mammalian HSD17B12 was identified as a homolog of YBR159w, a yeast microsomal enzyme. YBR159w is a 3-ketoacyl-CoA reductase (KAR) acting in the elongation of fatty acids. Accordingly, the recombinant HSD17B12 enzyme has demonstrated 3-ketoacyl-CoA reductase activity and involvement in long-chain fatty acid synthesis (Moon and Horton, 2003). In line with its function *in vitro*, the highest expression level of human *HSD17B12* has been detected in tissues associated with lipid metabolism, including the liver, muscle and kidney (Sakurai et al., 2006). In mice, *Hsd17b12* mRNA expression has additionally been detected in white and brown adipose tissue (Blanchard and Luu-The,

2007). Together with its function in fatty acid synthesis, the human HSD17B12 enzyme has been shown to convert estrone (E1) to estradiol (E2) in HEK 293 cells (Luu-The et al., 2006). The actual physiological function of the HSD17B12 enzyme has remained controversial, because the full *Hsd17b12* knockout mice die *in utero* and because fatty acid synthesis is imbalanced in mouse embryonic stem cells with reduced *Hsd17b12* expression (Rantakari et al., 2010). Additionally, the subfertility of the heterozygous *Hsd17b12* knockout mouse has been shown to be caused by defects in fatty acid synthesis rather than in steroid metabolism (Kemiläinen et al., 2016). On the other hand, the enzyme is proposed to be the major HSD17B enzyme for producing estradiol in postmenopausal women and adipose tissue (Bellemare et al., 2009; Luu-The et al., 2006). Supporting data was also reported by Paatela *et al*. (Paatela et al., 2016) whom observed higher mRNA expression levels of *HSD17B12* in adipose tissue of postmenopausal compared with premenopausal women.

2.2.1.8 HSD17B13

The function of HSD17B13, also known as short-chain dehydrogenase/reductase 9 (SCDR9), is only superficially known, but HSD17B13 has been shown to possess 78 % sequence identity with HSD17B11. Furthermore, the *HSD17B11* and *HSD17B13* genes are located in the same chromosomal loci in humans, mice and rats, suggesting co-evolution of the genes (Liu et al., 2007). HSD17B13 has been identified as a lipid-droplet (LD)-associated protein in hepatocytes, and HSD17B13 expression is restricted to the liver (Horiguchi et al., 2008), indicating a role for HSD17B13 in regulating LD metabolism. HSD17B13 has been found to be a pathogenic molecule in the development of non-alcoholic fatty liver disease (NAFLD) (Su et al., 2014).

2.3 Hydroxysteroid (17beta) dehydrogenase 1 (HSD17B1)

HSD17B1 was the first hydroxysteroid 17-beta dehydrogenase enzyme to be characterized. The protein was identified in the late 1950's (Langer and Engel, 1958) and purified from the human placenta soon after (Jarabak et al., 1962). Human HSD17B1 was then cloned, and the sequence was determined (Luu-The et al., 1989; Peltoketo et al., 1988). The structure and function of human HSD17B1 has been studied extensively, and current knowledge of the enzyme indicates that HSD17B1 is an important factor in local estrogen metabolism. HSD17B1 activity

in estrogen metabolism provides the basis of the importance of HSD17B1 in estrogen-dependent diseases and makes HSD17B1 a promising drug target for diseases such as endometriosis and breast cancer.

2.3.1 HSD17B1 gene and its genomic locus

2.3.1.1 Mouse Hsd17b1

The mouse *Hsd17b1* gene is located in chromosome 11 and, more precisely, at 11D;11.64.17cM. The *Hsd17b1* gene consists of 6 exons and 5 short introns and is approximately 2.1 kb in lenght (Yates *et al.*, 2016, Ensembl release 89, mouse GRCm38.p5). The neighboring genes in the locus include Coenzyme A synthase (*Coasy*) and alpha-N-acetyl-glucosaminidase (*Naglu*). *Coasy* is downstream of *Hsd17b1*, and *Naglu* is locating just 739 bp upstream from the transcriptional start site of *Hsd17b1*. (Yates *et al.*, 2016, Ensembl release 89, mouse GRCm38.p5). *Hsd17b1* encodes 1.4 kb transcript and the protein produced is 36 kDa in size. The regulatory regions for the mouse *Hsd17b1* gene have not been characterized in detail. However, in rodent ovaries, *Hsd17b1* is known to be regulated by cyclic adenosine monophosphate (cAMP)-mediated pathways and TGF-beta family members (Ghersevich et al., 1994a; Kaminski et al., 1997). Specifically, activin A of the TGF-beta family has been demonstrated to regulate *Hsd17b1* transcription via SMAD2, with downstream mediators that include activin receptor-like kinase 4 (ALK4, Bak et al., 2009). Additionally, luteinizing hormone (LH) has been shown to regulate *Hsd17b1*, as *Hsd17b1* expression is known to disappear from luteal cells after an LH surge in rodent ovaries (Stocco et al., 2007).

According to the WashU Epigenome Browser (http://epigenomegateway.wustl.edu/browser/), the mouse *Hsd17b1* gene contains three short interspersed nuclear elements (SINEs), called B4P, PB1D9 (Alu) and B2_Mm2 (Figure 3). Generally, these short repetitive, non-coding sequences are believed to be integrated into a complex regulatory network capable of modifying gene expression across the eukaryotic genome, *e.g.*, by affecting the 3D structure of DNA or by directly interacting with transcriptional repressors and activators (Goodrich and Kugel, 2006; Lee et al., 2016). SINE sequences belong to non-autonomous retrotransposons, as they commonly depend on enzymes encoded by long nuclear interspersed element (LINE) sequences for reverse transcription and retrotransposition. RNA polymerase III (pol III), which is known to transcribe ribosomal RNA and transfer RNA (tRNA), transcribes SINE sequences (Kramerov and Vassetzky, 2011).

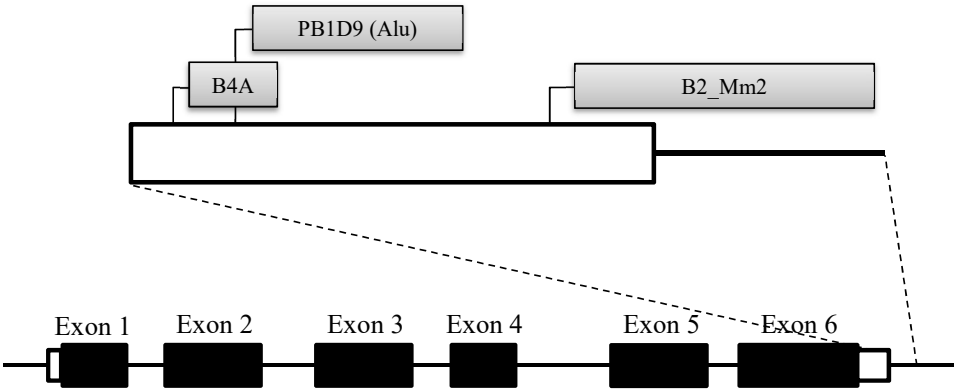

Figure 3. Schematic figure of the mouse *Hsd17b1* gene. Short interspersed nuclear repeat elements (SINEs) after the last exon, exon 6, in the 3' UTR region of the *Hsd17b1* gene.

2.3.1.2 Human HSD17B1 gene

The difference between the mouse and human genomic regions coding for HSD17B1 is that in humans, there is a pseudogene (*HSD17B1P1*) next to the *HSD17B1* gene (Figure 4, Luu-The *et al.*, 1990; Peltoketo, Isomaa and Vihko, 1992). The genes are located one after the other in chromosome 17, specifically at 17q21.2 (Luu-The et al., 1989; Peltoketo et al., 1988; Winqvist et al., 1990). *HSD17B1P1* is a pseudogene and is not translated into an active protein because it contains a premature stop codon (Luu-The et al., 1990; Peltoketo et al., 1992a). The function of the pseudogene, if any, has not been revealed. The protein-coding, 6 kb *HSD17B1* gene contains six exons and five short introns, and the gene produces two transcripts that are 1.3 and 4.9 kb in length (Yates *et al.*, 2016, Ensembl release 89, human GRCh38.p10). Human *HSD17B1* codes for a protein that is 328 amino acids long with a molecular weight of 34.9 kDa (Peltoketo *et al.*, 1988; Luu-The *et al.*, 1989; Yates *et al.*, 2016, Ensembl release 89, human GRCh38.p10). Regulation of human *HSD17B1* has been studied more extensively than that of mouse *Hsd17b1*. cAMP has been shown to decrease *HSD17B1* expression in primary cultures of human cytotrophoblasts and human granulosa cells, while in choriocarsinoma cells, cAMP increases *HSD17B1* expression (Tremblay et al., 1989). In addition, retinoic acids have been shown to induce *HSD17B1* gene expression

in cultured choriocarcinoma cells (Piao et al., 1995; Zhu et al., 2002), cytotrophoblast cells (Zhu et al., 2002) and cultured breast cancer cells (Piao et al., 1995). Reproductive hormones may also regulate *HSD17B1* expression, as FSH and estrogens induce *Hsd17b1* expression in rat granulosa cells via the cAMP pathway *in vitro* (Ghersevich et al., 1994b, 1994c). Progestin has also been shown to induce *HSD17B1* expression in T47D cells and in the endometrium (Mäentausta et al., 1993; Poutanen et al., 1990). The 5' UTR of *HSD17B1* includes typical elements of a promoter, such as a TATA box, a GC-rich area and an inverted CAAT element (Luu-The et al., 1990; Peltoketo et al., 1992b; Piao et al., 1995). The gene has also been shown to contain SINEs, including Alu sequences and cis-acting elements (Peltoketo et al., 1992b). Alu sequences are repeats in eukaryotic DNA that can have widespread influences on gene expression. The WashU Epigenome Browser (http://epigenomegateway.wustl.edu/browser/) shows that the 5' UTR of the human *HSD17B1* gene contains 3 Alu elements (AluSq, AluJr and AluJo) and one long terminal repeat (LTR) element. The 3' end also has one Alu sequence. All of these genetic elements can be involved in the regulation of gene expression, but these elements have not been studied in the context of regulating the expression of *HSD17B1* or neighboring genes.

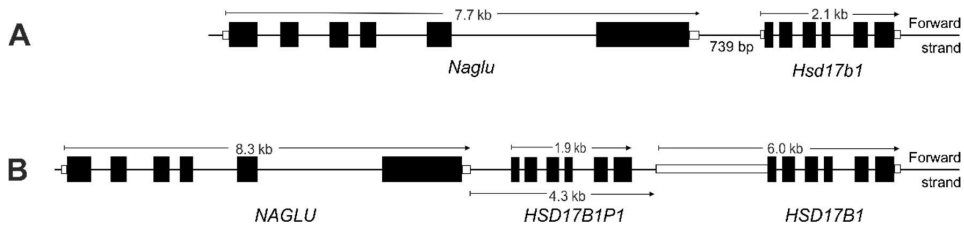

Figure 4. Genomic structures of mouse and human *NAGLU* and *HSD17B1* genes. A) Representation of the genomic loci of the mouse *Naglu* and *Hsd17b1* genes. B) Genomic loci of the human *NAGLU* and *HSD17B1* genes, which also show the pseudogene (*HSD17B1P1*) in between. Black boxes indicate exons.

2.3.2 Expression and function of Hsd17b1

Mouse *Hsd17b1* is expressed strongly in the ovaries. Moderate expression has been detected in the endometrium and adrenal glands of female mice, and minor expression has been detected in livers and testes of male mice and in the female brain (Nokelainen et al., 1996). Specific radiolabeling after *in situ* hybridization has been detected in the ovary, testis (seminiferous tubules but not Leydig cells), prostate (the epithelial cells bordering the acinar lumen), pituitary gland (both female and male, intermediate lobe) and skin (Pelletier et al., 2004). In the ovaries,

HSD17B1 is specifically localized to the granulosa cells of the growing follicle. However, the expression of *Hsd17b1* has not been detected in other steroidogenic or steroid target tissues, such as the uterus, mammary gland, vagina, kidney, adrenal gland, liver, lung or brain (Pelletier et al., 2004). Furthermore, the expression of mouse *Hsd17b1* has been clearly demonstrated in the seminiferous tubules of the developing testis (O'Shaughnessy et al., 2000; Pelletier et al., 2004), specifically in Sertoli cells (Hakkarainen *et al*. unpublished data). In humans, the highest expression level of *HSD17B1* was detected in the placenta. Interestingly, while the human placenta produces estrogens, the rodent placenta does not (Luu-The et al., 1990; Zhu et al., 2002). Like mouse *Hsd17b1*, human *HSD17B1* is expressed in the granulosa cells of the preantral follicles in adult ovaries (Ghersevich et al., 1994b; Luu-The et al., 1990; Vaskivuo et al., 2005). To a lesser extent, human *HSD17B1* is expressed in the endometrium (Šmuc et al., 2007), adipose tissue (Campbell et al., 2013; Paatela et al., 2016) and breast (Miettinen et al., 2000). However, a proportion of breast cancer specimens has been shown to exhibit strong *HSD17B1* expression (Miyoshi et al., 2001; Shibuya et al., 2008).

The expression profiles in both humans and mice indicate a steroidogenic function for the HSD17B1 enzyme. Both human and mouse HSD17B1 catalyze the activation of estrone (E1) to estradiol (E2) *in vitro* using NADH or NADPH as a cofactor (Nokelainen et al., 1996; Poutanen et al., 1993). E2 is the most potent estrogen and accordingly, local E1 to E2 conversion is known to play a crucial role in the development of estrogen-dependent diseases, such as breast cancer (Jansson, 2009; Miyoshi et al., 2001; Oduwole et al., 2004) and endometriosis (Colette et al., 2013; Huhtinen et al., 2012; Šmuc et al., 2007). The recombinant mouse HSD17B1 has also been shown to catalyze the reductive reaction of androstenedione (Adione) to testosterone (T) *in vitro* with the same catalytic efficiency as with estrogens (Nokelainen et al., 1996). The androgenic activity of human HSD17B1 in cultured cells is only 20 % of that obtained in the estrogen reaction (Poutanen et al., 1993; Puranen et al., 1997). HSD17B7 and HSD17B12 have also been shown to possess the ability to convert E1 to E2. The enzymatic properties of these reductive HSD17B enzymes regarding conversion of E1 to E2 have been compared, and the data have demonstrated that both HSD17B7 and HSD17B12 each possess a high Michaelis-Menten constant (Km) and low maximum velocity (Vmax), thereby differing from the markedly more efficient HSD17B1 enzyme (Luu-The et al., 2006; Törn et al., 2003).

2.4 N-acetyl-alpha-glucosaminidase (NAGLU)

2.4.1 Lysosomes and lysosomal enzymes

Lysosomes are membrane-bound organelles that are found in almost all animal cells. They appear as circular, ovoid or tubular cytoplasmic dense bodies that contain a matrix of proteins and are surrounded by membranes. Lysosomes are involved in processes such as cellular waste degradation, secretion, signaling and energy metabolism. Autophagy is a lysosomal degradation pathway for cytoplasmic materials and is activated during stress conditions, such as during viral infection. Thus, autophagy also influences regulation of cancer, physiological development and normal growth. There are three different, recognized autophagic routes: 1) macroautophagy, 2) microautophagy and 3) chaperone-mediated autophagy (CMA). In macroautophagy, a membrane is wrapped around cytoplasmic material, forming an autophagosome (Klionsky, 2005). Microautophagy is mediated by direct lysosomal engulfment of the cytoplasmic cargo (Kunzt et al., 2004). CMA is a type of autophagic pathway that is mediated by chaperone-dependent selection of soluble cytosolic proteins that are then targeted to lysosomes (Kaushik and Cuervo, 2012). Lysosomes contain over 50 acid hydrolases that can break down proteins, lipids, carbohydrates and nucleic acids in a process called degradation. The hydrolases are enclosed by a membrane containing highly glycosylated lysosomal membrane proteins, *e.g*. lysosomal-associated membrane protein 1 (LAMP1). One of these enzymes that has hydrolase activity is alpha-N-acetylglucosaminidase, NAGLU.

2.4.2 Naglu gene in mouse

The gene coding for mouse alpha-N-acetylglucosaminidase, *Naglu*, is located in chromosome 11:11D. The 3' end of the *Naglu* gene is only 739 bp upstream from the transcriptional start site of the *Hsd17b1* gene. The mouse *Naglu* gene consists of six exons and five introns and is approximately 8.2 kb in length. The protein-coding transcript is approximately 2.6 kb in length (Zhao et al., 1996) and the protein produced is 739 amino acids (aa) in size. Neither the promoter region nor regulation of the mouse *Naglu* gene have been studied.

2.4.3 NAGLU gene in human

The NAGLU protein has been purified from human tissue (von Figura, 1977; Sasaki et al., 1991; Weber et al., 1996) and comprehensively characterized. The *NAGLU* gene is located in chromosome 17q21.1. It extends over an 8.9 kb long sequence and consists of six exons and five introns. *NAGLU* encodes a 2.7 kb long cDNA, which produces a 743 amino acid protein with a predicted molecular mass of 82 kDa (Weber et al., 1996; Zhao et al., 1996). The mature and precursor forms of NAGLU have evident molecular weights of 77 and 80 kDa, respectively (Weber et al., 1996). The regulation of the human *NAGLU* gene has not been characterized, but most of the lysosomal genes are regulated by the binding of transcription factor EB (TFEB) binding to a motif known as the Coordinated Lysosomal Expression and Regulation (CLEAR) element (Sardiello et al., 2009).

2.4.4 Expression and function of Naglu

The mRNA of mouse *Naglu* is highly expressed in the liver, kidney, spleen and testis, with moderate expression also evident in various other tissues. In humans, *NAGLU* is expressed highly in the ovary, liver and peripheral blood leucocytes. In addition, quantifiable levels of the transcript have been found in the spleen, testis, prostate, small intestine, lung, kidney and placenta (Weber et al., 1996). NAGLU is a lysosomal enzyme that catalyzes the fifth step of degradation of glycosaminoglycans (GAGs) by removing of the N-acetylglucosamine (GlcNAc) residues that exist in heparan sulfate (HS, Figure 5) (von Figura, 1977). HS is a GAG that is composed mostly of a glucuronic acid linked to GlcNAc, and HS is localized to the extracellular matrix. Heparan sulfate binds to many protein ligands and regulates developmental processes, blood coagulation, angiogenesis, and tumor metastasis (Whitelock and Iozzo, 2005).

2.4.5 Defect in the function of NAGLU

Mutation of the human gene coding for NAGLU causes a disease called mucopolysaccharidoses (MPS) IIIB (Sanfilippo type B syndrome) (Andrade et al., 2015). Sanfilippo syndrome is one of a group of four MPS disorders (A, B, C and D) that are caused by deficiencies in enzymes that are involved in heparan sulfate (HS) degradation. Due to the NAGLU defect, partially degraded GAGs accumulate in lysosomes, causing lysosomal hypertrophy, cell death and, ultimately, organ dysfunction. MPS IIIB patients have behavioral problems, symptoms of mental retar-

dation, and somatic manifestations that are highly variable among different phenotypes, and the disease outcome is premature death, usually in the second decade of the patient's life (Beesley et al., 2005).

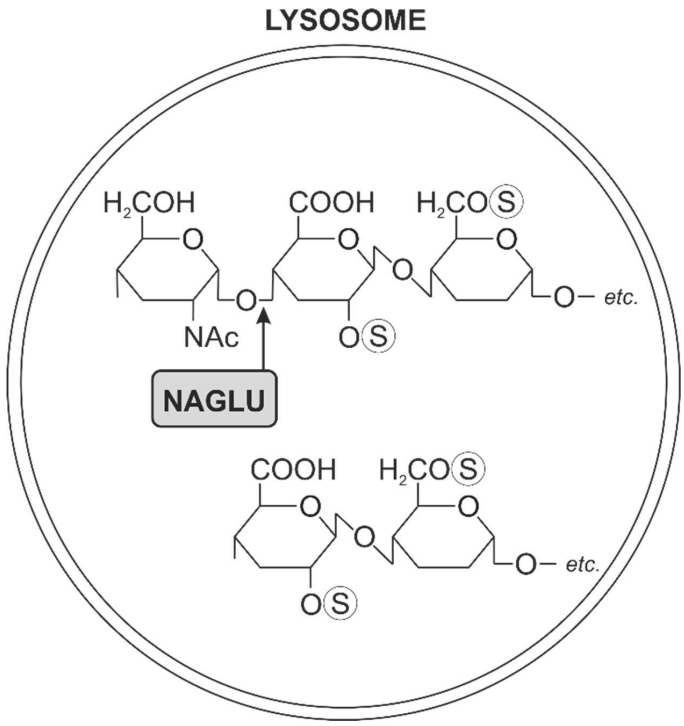

Figure 5. NAGLU function in lysosome. NAGLU enzyme degrades heparan sulfate by hydrolysis of terminal N-acetyl-D-glucosamine residues in N-acetyl-alpha-D-glucosaminides.

There are several mouse models of for MPS disorders that have been generated by targeted disruption of the corresponding gene. The pathological and biochemical features of these animal models are mostly similar to those of corresponding humans with these syndromes (Bhaumik et al., 1999; Li et al., 1999; Marcó et al., 2016). The Naglu-Neo mice were generated by disrupting exon 6 of *Naglu*, and the disruption resulted in total inactivity of the NAGLU enzyme (Li et al., 1999). These mice demonstrated massive increase of GAGs in the liver and kidney and major increase in certain brain gangliosides. As a consequence, there was a vacuolization in the macrophages and neurons of the mutant mice occured. In addition, the behavioral changes in the Naglu-Neo mice resembled those of patients with the human Sanfilippo syndrome type B. The Naglu-Neo mice also had greatly distended urinary bladders from the age of 6 months (Li et al., 1999).

2.5 Female reproductive system

Fertility in all mammals depends on a functional hypothalamic-pituitary-gonadal axis. In females, gonadotropin-releasing hormone (GnRH) released from the hypothalamus reaches the anterior pituitary, from which luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are secreted. Via the blood circulation, these hormones stimulate ovarian steroidogenesis and ovulation. Ovarian follicles then secrete estrogens that reach the hypothalamus and exert feedback effects to the hypothalamus and pituitary gland to maintain homeostasis within the axis (Levine, 2015).

2.5.1.1 Puberty and estrous cycle

Puberty in females consists of many endocrine and physiological changes. Increased production of estradiol and progesterone is directly caused by sexual maturation. The first observable signs of puberty in female mice are estrogen-dependent, including vaginal opening and a cornified vaginal smear. In mice, the vaginal opening is not necessarily a sign of puberty onset, but vaginal opening is an indication of an increased estradiol level (Rodriguez et al., 1997). Commonly, the vaginal opening occurs in mice that are approximately 26 days old. The majority of females ovulate for the first time between the ages of 6 to 8 weeks (Nelson et al., 1982). After first ovulation, the estrous cycle begins.

First estrus correlates with the ability of a female mouse to become pregnant. The estrous cycle can be determined by daily collection and analysis of vaginal smears. The normal estrous cycle in mice is four to six days long and consists of proestrus, estrus, metestrus and diestrus phases (Figure 6). The proestrus stage begins when a new patch of eggs reaches maturity. Proestrus can be detected with vaginal smears, which show high levels of rounded, nucleated epithelial cells. At this stage, the E2 level increases, and consequently, the LH and FSH surges follow, leading to ovulation (Caligioni, 2009). Estrus begins with the ovulation of mature oocytes, and females are maximally receptive for males at this stage. Estrus can be detected by observing fragments of epithelial cells that appear flat and irregularly shaped in the vaginal smear. Estradiol remains elevated throughout the estrus phase (Walmer et al., 1992). Estrus phase is followed by the metestrus phase, which is characterized by movement of the mature eggs into the uterus, and closing of the vagina, which has been open since proestrus and estrus phases. A mixture of cornified and nuclear epithelial cells and leukocytes are evident in the vaginal smear during the metestrus phase. If pregnancy does not occur at this point, the diestrus phase immediately follows immediately metestrus and the vaginal smear becomes full of

leukocytes (Levine, 2015). During metestrus, the E2 level is low, and during diestrus the E2 levels start to increase (Walmer et al., 1992).

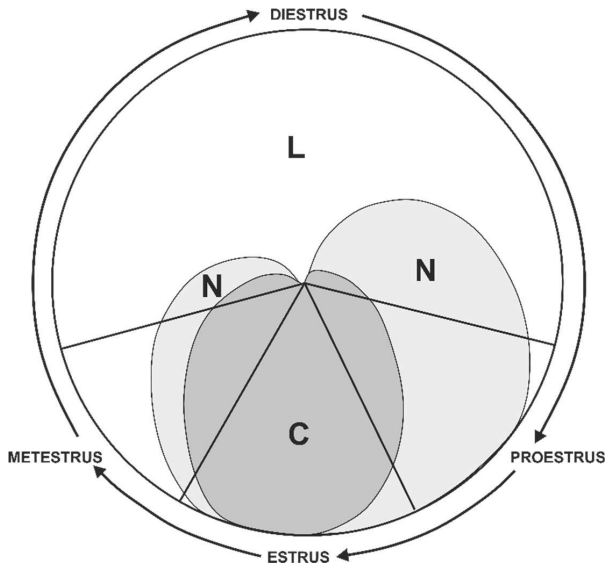

Figure 6. Estrous cycle stages. Schematic diagram of cell types that are present in vaginal smears during four phases of estrous cycle. The lines inside the circle indicate the points where estrous phase changes, and the size of each sector is an estimation of the length of the stage. The total cycle takes approximately 4–6 days. L = leukocytes, N = nucleated epithelial cells and C = cornified epithelial cells (Modified from Byers *et al.*, 2012)

2.5.1.2 Pregnancy and pseudopregnancy

Upon successful copulation during the estrus phase, hormonal changes take place to prepare the uterus for implantation and pregnancy. The hormones derived from the follicles program the secretion of gonadotropins to prepare the reproductive tract for conception, implantation and pregnancy. The fertilized zygote forms and implants into the uterus, which, in the mouse, occurs around embryonic day (E) 4. Ovarian estrogen and progesterone act in collaboration with locally produced signaling molecules during the implantation event (Hemberger and Dean, 2014). Pregnancy in mice lasts 19-21 days. In mice, mating triggers the formation of a persistent *corpus luteum* (CL), and the CL of pregnancy forms (Stocco et al., 2007) regardless of the fertility of the male. When female mice are mated with sterile males, mating is followed by a period of extended luteal function lasting for 12–

14 days. This phase is called pseudopregnancy, and during this period, high prolactin secretion from the pituitary gland occurs in response to the mating stimulus (Freeman and Neill, 1972; Freeman et al., 1974). Prolactin secretion results in persistence of a functional CL for approximately 10 days under the assumption that a pregnancy will follow (Smith et al., 1975). Prolactin inhibits expression of 20α-hydroxysteroid dehydrogenase, thereby preventing catabolism of progesterone to 20α-OH-progesterone (Zhong et al., 1997). As a result of these changes, the uterus becomes receptive to implantation. Pseudopregnant female mice can also be used for successful implantation and development of fostered embryos, such as those applied in the generation of GM mice.

2.5.2 Function of the ovaries

2.5.2.1 Follicular life cycle and steroidogenesis in the ovaries

There are many cellular components in follicles that interact together to secrete sex steroid hormones and regulate the development of oocytes. The follicular life cycle starts from primordial oocytes, which consist of late diploid primary oocytes surrounded by a single layer of flattened granulosa cells. The growth of primordial follicles starts during the fetal development in both humans and rodents and occurs continuously until menopause in humans; mice do not experience menopause. During follicular growth, granulosa cells proliferate and go through morphological changes from a flattened to cuboidal shape (Van Deerlin et al., 1997). In addition, oocyte growth and zona pellucida formation occurs at the initiation stage. When the follicle reaches the primary follicle stage, theca cells, including the theca interna and theca externa, start to surround the granulosa cells and oocyte. Formation of the primary follicle is followed by formation of the secondary follicle, in which the granulosa cells develop receptors for androgens, estrogens and FSH and form couples via gap junctions (Gittens et al., 2003). In secondary follicles, theca cells start to express LH receptors and become the capable of producing steroid hormones. FSH stimulation promotes the formation of antral follicles from secondary follicles. The antral follicle grows and becomes a Graafian follicle, which is ready for ovulation. The antrum serves as an environment where the cumulus–oocyte complex grows, matures and ovulates. Expansion of the antrum requires the influx of water by an osmotic gradient that is stimulated by granulosa cells that actively transport ions or by GAG hydrolysis in the antrum. GAGs might increase the osmolarity of the follicular fluid and support the influx of water (Bellin et al., 1986; Clarke et al., 2006; Rodgers et al., 2001; Strauss and Williams, 2014). After ovulation, the ruptured follicle is restructured into a CL. Under the influence of LH,

theca cells luteinize into small luteal cells, and granulosa cells luteinize into large luteal cells. Progesterone is synthesized in both large and small luteal cells. One very prominent feature of CL development is the establishment of a rich vascular network from surrounding vessels (Stocco et al., 2007).

Ovaries produce the female hormones, namely, estrogens and progesterone, via enzymes that are under transcriptional control of pituitary LH and FSH. There are three gonadal estrogens: E1, E2 and estriol (E3), from which estradiol is the most biologically active. E2 production in the ovaries occurs in the developing follicles, specifically in the granulosa cells and theca cells. Theca cells convert cholesterol to pregnenolone, and pregnenolone is then converted to androstenedione (Adione). The granulosa cells complete the conversion of androgens to estrogens; aromatase (CYP19A1) converts the 17-keto androgen to estrone, and thereafter, the HSD17B1 enzyme converts E1 to E2. After ovulation, the CL produces mostly progesterone (P4) due to the high expression levels of CYP11A1 and HSD3B2. The amount of progesterone depends not only on the amount of synthesis in the luteal cells but also on the 20alpha-HSD enzyme that catabolizes progesterone into inactive progestin. Human *HSD17B1* and mouse *Hsd17b1* are highly expressed in the granulosa cells of the developing follicle, but expression disappears after LH release and luteinization (Stocco et al., 2007).

2.5.2.2 Lysosomal activity in the ovary

Ovary is a dynamic organ that undergoes cycles of growth and regression with the help of lysosomes. Some of the follicles grow and reach nearly mature states, but they do not ovulate. These atretic follicles, along with the regressing CL, are degraded by autophagic vacuoles (Weckman et al., 2013). In addition to digesting the subcellular contents of ovaries, autophagy is required for germ cell survival during ovarian development, as the loss of two important autophagic factors, *Becn1* or *Atg7,* results in premature loss of female germ cells (Gawriluk et al., 2011). Lysosomal activity is also involved in granulosa cell survival and cell death (Duerrschmidt et al., 2006; Gaytán et al., 2008; Serke et al., 2009). In addition, it has been suggested that lysosomes are involved in decreasing levels of gonadotropin receptors in the plasma membranes of ovarian cells, which modulates the action of gonadotropic hormones (Chen et al., 1977). It has been observed that the *Naglu* knockout mice have reduced fertility after 6 months of age (Li et al., 1999; Ohmi et al., 2009). However, fertility statuses for patients with and animal models of lysosomal disorder have not been reported otherwise.

2.6 Metabolic processes in adipose tissue and liver

2.6.1 White adipose tissue

Two different kinds of adipose tissues exist: white adipose tissue (WAT) and brown adipose tissue (BAT). Most of the fat in adult mammals is WAT, and WAT is an important site for lipid metabolism, insulin signaling and endocrine actions. BAT is mainly involved in the control of the thermogenesis that is facilitated by mitochondrial uncoupling protein-1 (UCP1) (Sarjeant and Stephens, 2012). White adipose tissue mainly consists of mature adipocytes. The cells appear morphologically as one big lipid droplet, cell membrane and flattened nucleus close to the cell membrane. In addition, adipose tissue contains different cell types, including endothelial cells, blood cells, preadipocytes and macrophages (Geloen et al., 1989). The central function of adipose tissue is to store energy as fat (mainly as triglycerides) and release energy when needed, but adipose tissue also functions as an active endocrine organ that responds to metabolic signals and that secretes many humoral factors, such as leptin and adiponectin (Ottaviani et al., 2011).

2.6.1.1 Sex steroids in adipose tissue

Adipose tissue is an active endocrine organ that is known to be an important site for extragonadal steroid biosynthesis and metabolism. Particularly in women after menopause, E2 is produced in peripheral tissues, and much of this production occurs in adipose tissue (Labrie, 2015). It has been demonstrated that human adipocytes can take in and hydrolyze dehydroepiandrosterone sulfate (DHEAS) to dehydroepiandrosterone (DHEA) (Dalla Valle et al., 2006). The hormonally inactive DHEA is then used as a precursor to generate T or E2, according to the specific needs of the adipose tissue or other tissues (Labrie, 2015), via the expression of steroidogenic enzymes (Figure 7; Li et al., 2015; Tchernof et al., 2015). Great differences in the expression levels of these steroidogenic enzymes are evident between premenopausal and postmenopausal women and between different fat depots. For example, *HSD17B12* expression is higher in subcutaneous adipose tissue of postmenopausal women than in premenopausal women, which is similar to *CYP19A1* (Paatela et al., 2016). Furthermore, increases in CYP19A1 activity have been measured in subcutaneous fat relative to visceral fat, especially in females (Corbould et al., 2002; McTernan et al., 2002). The expression of *HSD17B7* has been found to be higher in visceral fat than in subcutaneous fat in women (Paatela et al., 2016). The expression of *HSD17B1* has been detected in the visceral and subcutaneous adipose tissues of both premenopausal and postmenopausal women.

No clear differences expression-level differences have been observed between depots or between premenopause and postmenopause (Paatela et al., 2016), but a significant decrease in expression has been associated with notable weight loss in postmenopausal women (Campbell et al., 2013). HSD17B activity has been detected in white adipose tissue for decades (Bleau et al., 1974), and the expression level of *HSD17B12* has been shown to be much higher than the expression levels of *HSD17B1* and *HSD17B7*, indicating that HSD17B12 would be the most important enzyme for producing estradiol in adipose tissue (Bellemare et al., 2009).

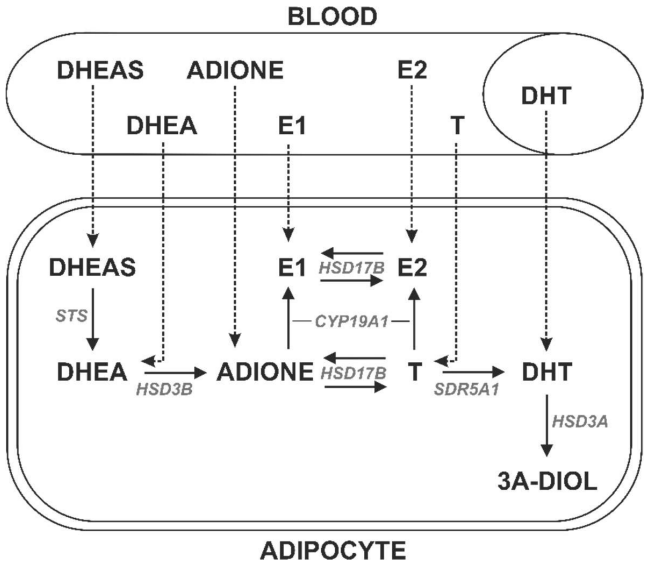

Figure 7. Steroid metabolism in adipose tissue. Steroid precursors from blood circulation can be taken up by adipocytes and further converted to active estrogens or androgens by various steroid-converting enzymes (Modified from Li et al., 2015).

While adipose tissue acts as an endocrine organ, male and female hormones control the adipose tissue distribution and function (Mauvais-Jarvis, 2011; Mauvais-Jarvis et al., 2013). Consequently, differences exist between women and men. In mean, the android fat distribution causes fat accumulation in the abdomen, while in women, fat tends to accumulate in the gluteal-femoral area in women. In addition, abdominal adipose tissue tends to accumulate in the visceral area to a greater extent in men than in women (Kuk et al., 2005; Pulit et al., 2017). Sex steroids can also contribute adipocyte differentiation, as *in vitro* studies have shown that estradiol inhibits adipocyte differentiation (Okazaki et al., 2002).

2.6.1.2 Lysosomal activity in adipose tissue

Macroautophagy is a lysosomal pathway for cytosolic degradation. In this process, cell organelles and portions of the cytosol are isolated into double-membrane vesicles, called autophagosomes, which move to the vicinity of lysosomes for fusion and content degradation. Lipid droplets have been identified as one of the substrates of autophagosomes in which breakdown of lipid components occurs (Dong and Czaja, 2011). In addition to regulating the nutrient supply and cell death, autophagy plays a role in cellular development and differentiation (Singh et al., 2009). Adipocytes differentiate from mesenchymal precursors and undergo a remodeling process to become fat depots. Remodeling consists first of an increase in mitochondrial biogenesis, followed by a massive reduction in the number of mitochondria in the cell; the mitochondria are mostly replaced by lipid droplets. Autophagosomes engulf mitochondria during adipogenesis (Goldman et al., 2011). Inhibition of autophagy *in vitro* and *in vivo* has shown an effect on the differentiation of adipocytes and the function of these cells, as inhibition blocks white adipocyte differentiation *in vitro* and decreases the WAT mass markedly *in vivo*. Decreased lipid storage occurs in parallel with these changes (Baerga et al., 2009; Singh et al., 2009). Decreased adipose mass is a universal feature of lysosomal disorders. Accordingly, deficiency in the NAGLU enzyme has been shown to cause profoundly decreased adiposity in mice (Woloszynek et al., 2007). The mechanism behind the metabolic phenotype is not clear, but the phenotype is not a result of reduced caloric intake or an increased metabolic rate.

2.6.2 The structure and function of the liver

The liver is a structurally and functionally heterogeneous tissue, which, in mice, has four different liver lobes: median, left, right and caudate. The functional unit in the liver is the lobule, and the lobule consists of a central vein in the middle, of portal triads in the peripheral region and of hepatocytes and liver sinusoid endothelial cells in between. The portal triads include the portal vein, arteria and bile duct (Rogers and Dintzis, 2012). The liver has thousands of functions, including the uptake of amino acids, carbohydrates, bile acids, cholesterol, proteins and lipids for storage and metabolism. Thereafter, the metabolized compounds are released into bile and/or blood (Rogers and Dintzis, 2012). Furthermore, the liver controls the blood volume and is the main site of defense against foreign macromolecules (Malarkey et al., 2005).

Many metabolic diseases, such as obesity, metabolic syndrome and lipodystrophy, can result in lipid accumulation in the liver. This pathological state is called fatty

liver or liver steatosis. Obesity-related steatosis, or non-alcoholic fatty liver disease (NAFLD) in particular, is frequently associated with insulin resistance and type 2 diabetes mellitus. Increase of NAFLD is almost epidemic following the global obesity increase (Fabbrini et al., 2010; Gluchowski et al., 2017). Simple hepatic steatosis, without ballooned hepatocytes and lobular inflammation, is considered to be a benign condition without an increase in liver-related morbidity and mortality, but non-alcoholic steatohepatitis (NASH), with added necroinflammation and fibrosis, may progress to cirrhosis (Suriawinata and Thung, 2011). Lipodystrophy is a disorder of the adipose tissue characterized by a partial or total absence of body fat. Lipodystrophy patients tend to develop insulin resistance, diabetes, hypertriglyceridemia and fatty livers (Vigouroux et al., 2011).

2.6.2.1 Steroids in liver function

In mice many hepatic genes show sexual dimorphism (~70 %). The largest changes (over 3-fold) in gene expression between females and males are observed in genes involved in steroid and lipid metabolism (Yang et al., 2006). Sex steroids regulate lipid, cholesterol and glucose homeostasis in the liver, and therefore, they might regulate the development of hepatic steatosis. Estradiol decreases gluconeogenesis, lipogenesis and fatty acid uptake, while it enhances glucose catabolism, lipolysis and cholesterol secretion in the livers of females (Foryst-Ludwig and Kintscher, 2010; Shen and Shi, 2015). Thus, estrogens may protect against lipid accumulation in the liver and further protect against NAFLD (Polyzos et al., 2013). In males, testosterone works via androgen receptors, increasing insulin receptor expression and glycogen synthesis. Furthermore, androgens decrease lipogenesis and glucose uptake and promote cholesterol storage in the liver (Kelly et al., 2014; Lin et al., 2008; Livingstone et al., 2015; Shen and Shi, 2015).

2.6.2.2 Lysosomal activity in liver function

Lysosomes are abundant in hepatocytes, as hepatocytes are secretory cells. The turnover of different energy stores and elimination of dysfunctional mitochondria by autophagy helps to maintain energy balance in the liver. Lysosomal activity is also needed for cellular quality control, which involves eliminating altered proteins and organelles; otherwise hepatocyte toxicity occurs. Liver autophagy is also activated as a defense against hepatic damage resulting from various factors, including oxidative stress (Ueno and Komatsu, 2017). In addition to demonstrating active breakdown of energy stores, lysosomes control liver metabolism by degrading limiting enzymes in distinct metabolic pathways (Cuervo et al., 1995). Liver-specific

autophagy also plays a role in blood glucose regulation (Ezaki et al., 2011), and upregulation of autophagy has been shown to improve insulin sensitivity (Rautou et al., 2011). Lysosomal activity can have an effect on lipid accumulation in the liver as the liver lysosomal lipase is needed for degradation of lipid droplets (lipophagy) (Singh and Cuervo, 2012). Altogether, lysosomal activity, especially autophagy, plays a protective role in most liver diseases and is thus a potential drug target (Rautou et al., 2011).

3 AIMS OF THE STUDY

In the present thesis, I have focused on the HSD17B1 enzyme, which has been shown to catalyze the reaction that activates E1 to E2. In line with its activity, the *Hsd17b1* gene is highly expressed in ovarian granulosa cells during follicular development in mice. However, *Hsd17b1* is also expressed in various extragonadal tissues, indicating other unknown functions for the enzyme.

The general aim of the thesis was to further understand the role of HSD17B1 in regulating the extent of estrogen actions by applying knockout mouse models. During the study, the following specific aims were addressed:

AIM 1: To clarify the role of HSD17B1 in estradiol biosynthesis and in the regulation of the estrogen actions in sex-steroid target tissues by analyzing the gonadal and extra gonadal tissues of Hsd17b1-LacZ/Neo female mice.

AIM 2: To elucidate the role of HSD17B1 in lipid metabolism by analyzing the metabolic phenotypes of Hsd17b1-LacZ/Neo male mice.

AIM 3: To examine how the replacement of the *Hsd17b1* gene with a LacZ/Neo cassette affects function of neighboring genes.

4 MATERIALS AND METHODS

4.1 Genetically modified mouse lines used in the study (I, II)

This thesis is based on studies performed with several mouse models: Hsd17b1-LacZ/Neo, Hsd17b1-lacZ, Hsd17b1-LacZ/Neo X HSD17B1TG and Naglu-Neo. In all experiments, wild type (WT) littermates were used as controls. Hsd17b1-LacZ/Neo mice, where the *Hsd17b1* gene is replaced with LacZ/Neo cassette, were generated with embryonic stem (ES) cells in the C57BL/6N background. In the targeted ES cells, which were obtained from the Knockout Mouse Project (KOMP) Repository (http://www.komp.org/), the whole coding region of the *Hsd17b1* gene was replaced with a *lacZ* reporter gene followed by the *neomycin* (*neo*) sequence under the *human ubiquitin 1* (*hUBC*) promoter. Proper targeting in the ES cells was confirmed by sequencing, and the clones were injected into the blastocysts of C57BL/6N origin. Chimeric male pups were born and bred with C57BL/6N females to establish germline transmission. Furthermore, pups were genotyped with PCR primers detecting WT (SU and exon1as1) and the targeted *Hsd17b1* allele (SU and LacZRev) (Table 1).

In Hsd17b1-LacZ mice, we removed the antibiotic resistance gene (*neo*) from Hsd17b1-LacZ/Neo mice by crossing them with Rosa26 transgenic mice (Ventura et al., 2007). The Rosa26 mice ubiquitously express Cre recombinase under the Rosa26 promoter, and crossing these mice with the Hsd17b1-LacZ/Neo mice resulted in deletion of the *neo* gene from the targeted *Hsd17b1* locus. The deletion was confirmed by genotyping PCR with primers SD and SV40, which are presented in Table 1. The Hsd17b1-LacZ/Neo mice were also crossed with transgenic mice constitutively expressing the human *HSD17B1* gene under the chicken beta-actin promoter (Saloniemi et al., 2007). The transgenic allele was identified with PCR primers Fw2 and ex32, which are presented in Table 1. Furthermore, the mice with the disrupted *Naglu* gene, namely, the Naglu-Neo mice, were studied in this thesis (Li et al., 1999). The Naglu-Neo mice were obtained from The Jackson Laboratory (Bar Harbour, ME, USA). In the Naglu-Neo mice, the last exon, exon 6, had a *neo* insertion with the *Pgk1* promoter, and the genotyping primers for this mouse line (NagluF, NagluWTR and NagluKOR) are presented in Table 1.

All mice used in these studies were housed under controlled conditions (12 h light / 12 h darkness at 21 ± 3°C and humidity at 55 ± 15 %) with *ad libitum* access to tap water and diet at the Central Animal Laboratory of University of Turku. All mouse models were provided with a normal diet (ND, Soy-free SDS RM3, 4 % fat, Special Diet Service; Witham Essex, UK), additionally, the Hsd17b1-LacZ/Neo males were challenged for 12 weeks with a high fat diet (HFD, C 1090

- 45 obesity-inducing diet with w/45 %, 22 % fat, Altromin, Lage, Germany). The animal experiments in this study were approved by the national Animal Ethics Board in Finland.

Table 1. Primer sequences used to genotype the mouse models

Mouse model	Allele	Primer	Sequence
Hsd17b1-LacZ/Neo	Genomic	SU	5´-CAACCCATCCCTTGATCAAC-3´
	Genomic	exon1as1	5´-CATCATCGTGCTTACCTCTG-3´
	Targeted	LacZRev	5´-GTCTGTCCTAGCTTCCTCACTG-3´
Hsd17b1-LacZ	Targeted	SV40	5´-GTTTATTGCAGCTTATAATG-3´
	Targeted	SD	5´-AACCCTTAGCCTAGCACCTTG-3´
HSD17B1TG	Targeted	Fw2	5´-CTTCAGATCCATCCCAGAGC-3´
	Targeted	Ex32	5´-GCCCAGGCCTGCGTTACAC-3´
Naglu-Neo	Genomic	NagluF	5´-GTCGTCTCCTGGTTCTGGAG-3´
	Genomic	NagluWTR	5´-ACCACTTCATTCTGGCCAAT-3´
	Targeted	NagluKOR	5´-TGGATGTGGAATGTGTGCGAG-3´

4.2 Tests for reproductive performance of Hsd17b1-LacZ/Neo and Naglu-Neo females (I, II)

The pubertal onsets of the Hsd17b1-LacZ/Neo female mice were studied by daily monitoring of the vaginal opening at 20 days of age to the day of vaginal opening or to 35 days of age. Furthermore, to determine the estrous cycles of the Hsd17b1-LacZ/Neo and Naglu-Neo mice, cytological analysis of vaginal smears was performed. The vaginas of adult mice were aspirated in the morning with a drop of phosphate-buffered saline (PBS) for 21 days. The smear was pipetted onto the objective glass and allowed to air dry. Thereafter, the vaginal smears were fixed with 96 % ethanol, stained with Mayer's hematoxylin (Histolab Products, Gothenburg, Sweden) and washed in tap water. The estrous cycle phase was determined for each day by analyzing the cell type under a microscope.

For elucidating fertility in the Hsd17b1-LacZ/Neo and Naglu-Neo females, the female mice were mated with WT male mice of known fertility for 60 days, and fertility was compared to that of the WT female littermate. The vaginal plug was checked every morning. After pups were born, the litter size and sex were verified. In addition, a pseudopregnancy tests were performed for the Hsd17b1-LacZ/Neo mice by breeding the knockout and WT females with infertile male mice for 31 days. The vaginal plug was checked every morning, and the length of the pseudopregnancy was determined by assessing the time between the mating plugs, as no copulation is supposed to occur during pregnancy initiation.

4.3 Analyses of mRNA expression (I, II)

For RNA isolation, the mice were sacrificed with CO_2 asphyxiation followed by blood withdrawal from the heart. Death was confirmed by cervical dislocation. The tissues were dissected, weighed rapidly, and snap-frozen with liquid nitrogen. Total RNA was isolated from the snap-frozen tissues using the TriSure reagent (Bioline, Inc., Tauton, MA, USA), in line with the manufacturer's instructions. The extracted total RNA was finally dissolved in nuclease-free water. The quality and quantity of the isolated RNA was determined with a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and by agarose gel electrophoresis. RNA samples were treated with either the DNase Amplification Grade Kit (Invitrogen, Life Technologies, Paisley, UK) or DNase I Amplification Grade (Sigma, Saint Louis, MO, USA) and used for cDNA synthesis done with the SensiFAST cDNA Synthesis kit (Bioline Inc., Tauton, MA, USA). The studied genes and their primer sequences are presented in Table 2. Quantitative RT-PCR was performed with SYBR Green (Bio-Rad, Hercules, CA, USA) and with the CFX96 real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA, USA). All genes related to steroidogenesis were analyzed using 6 Hsd17b1-LacZ/Neo and 6 WT ovaries in the proestrus, estrus and pseudopregnant stages. *Naglu* was additionally analyzed in the liver, kidney, adrenal gland, gonadal WAT, BAT, ovary, uterus, testis and epididymis of the Hsd17b1-LacZ/Neo, Hsd17b1-LacZ and Hsd17b1-LacZ/Neo X HSD17B1TG mice. The mRNA levels of the lipogenic enzymes, namely, *Acaca* and *Fasn*, were analyzed in the liver, and the mRNA levels of *Ucp1* were analyzed in the adipose tissues of the Hsd17b1-LacZ/Neo mice. The mRNA expression levels of the cholesterogenic enzymes (*Hmgcr*, *Cyp51*, *Nsdhl*, *Hsd17b7*, *Dhcr24* and *Dhc7*) were analyzed in the WT ovaries of normal cycling and pregnant females. The expression levels of the different genes were normalized to *beta-actin* (*Actb*) or *Ribosomal gene 19* (*L19*).

4.4 HSD17B activity in Hsd17b1-LacZ/Neo ovaries (I)

Intraovarian HSD17B activity was determined for both WT and Hsd17b1-LacZ/Neo mice using High Performance Liquid Chromatography (HPLC), connected to an online β-counter (PerkinElmer, Waltham, MA, USA; Waters S.A.S, Saint-Quentin, France). Briefly, the ovaries of three-month-old WT and Hsd17b1-LacZ/Neo mice were homogenized in 10 mM KH_2PO_4 pH 7.4, 1 mM EDTA, 20 % glycerol (Fisher Scientific, Thermo Fisher Scientific, Vantaa, Finland) and 0.01 % BSA (Sigma-Aldrich, St. Louis, MO, USA). Protein concentrations of tissue homogenates were quantified by using the Pierce™ BCA Protein Assay kit

(Thermo Scientific Pierce, Thermo Fisher Scientific, Rockford, IL, USA) according to the manufacturer's instructions. Then, the homogenate, including 3 µg of protein, was mixed with [3H]-E1, whose final concentration for E1 was 33 µM (~ 500 000 cpm, PerkinElmer, Life Sciences, Boston MA, USA).

Table 2. Sequences of primers used for qRT-PCR

Gene	Forward primer	Reverse primer
Hsd17b1	5′-CAACCCATCCCTTGATCAAC-3′	5´-CACCCACAGCGTTCAATTCA-3´
Hsd17b2	5′-CATCATCGTGCTTACCTCTG-3′	5´-CCTTGGACTTTCTAAGTAGAGGCA-3´
Hsd17b7	5′-GTCTGTCCTAGCTTCCTCACTG-3′	5´-GGCAGGATTCCAGCATTCAG-3´
Hsd17b12	5′-GTTTATTGCAGCTTATAATG-3′	5´-TTTCTTGATGGTGTTGTCCAAG-3´
Star	5′-AACCCTTAGCCTAGCACCTTG-3′	5´-CCGTGTCTTTTCCAATCCTCTG-3´
Cyp11a1	5′-CTTCAGATCCATCCCAGAGC-3′	5´-CGCATGAGAAGAGTATCGACGCATC-3´
Cyp17a1	5′-GCCCAGGCCTGCGTTACAC-3′	5´-AGGATTGTGCACCAGGAAAG-3´
20alpha-Hsd	5′-GTCGTCTCCTGGTTCTGGAG-3′	5´-TCTGGACGATGGGAAGTTGA-3´
Hsd3b1	5′-ACCACTTCATTCTGGCCAAT-3′	5´-GTGGCCATTCAGGACGAT-3´
Cyp19a1	5′-TGGATGTGGAATGTGTGCGAG-3′	5´-GCCGTCAATTACGTCATCCT-3´
Esr1	5´-TTGACAAGAACCGGAGGAAG-3´	5´-ATAGATCATGGGCGGTTCAG-3´
Esr2	5´-GACAAGAACCGGCGTAAAAG-3´	5´-GGACGGCTCACTAGCACATT-3´
Lhcgr	5´-GCCCTGAGCCCTGCGACTGC-3´	5´-AAAGCGTTCCCTGGTATGGTGGTT-3´
Fshr	5´-TGTCATTGCTCTAACAGGGTCT-3´	5´-TGGTGAGCACAAATCTCAGTTC-3´
Pgr	5´-CTCCGGGACCGAACAGAGT-3´	5´-ACAACAACCCTTTGGTAGCAG-3´
Prlr	5´-CCACAAATGTCGTTCCCCTG-3´	5´-GAATTGGGGCCACTGGTTTT-3´
Gdf-9	5´-GCTCTATAAGACGTATGCTACC-3´	5´-CAGAGTGTATAGCAAGACCGAT-3´
Ccdn2	5´-GAGTGGGAACTGGTAGTGTTG-3´	5´-CGCACAGAGCGATGAAGGT-3´
Ucp1	5´-AGGCTTCCAGTACCATTAGGT-3´	5´-CTGAGTGAGGCAAAGCTGATTT-3´
Acaca	5´-ATCCGCCTCTTCCTGACAAA-3´	5´-CCTAAGGACTGTGCCTGGAA-3´
Fasn	5´-TCTGTGCCCGTCGTCTATAC-3´	5´- GGAGGTATGCTCGCTTCTCT-3´
Naglu	5´-TCCAACAGCACGAGTTTGAG-3´	5´-CTGCGATGGCTAATCTGTCA- 3´
luciferase	5´-GATCAAAGCAATAGTTCACG-3´	5´-ATTTTTGATGGCAACATGGT-3´
Hmgcr	5´-TGGAGATCATGTGCTGCTTC-3´	5´-GCGACTATGAGCGTGAACAA-3´
Cyp51	5´-ATTGCCCGTATTCTGTGCTC-3´	5´-TGGCCTCAAATTCTCAATCC-3´
Nsdhl	5´-GATGCCAACGACCCTAAGAA-3´	5´-AACCACATTCTCCACGAAGG-3´
Dhcr24	5´-CATCTTCCGCTACCTCTTCG-3´	5´-CTCTGCTTCATCTCCCTTGG-3´
Dhcr7	5´-CGCTCCCAAAGTCAAGAGTC-3´	5´-GTGTCTTGGCCCAAATGTCT-3´
L19	5´-GGACAGAGTCTTGATGATCTC-3´	5´-CTGAAGGTCAAAGGGAATGTG - 3´
Actb	5´-CGTGGGCCGCCCTAGGCACCA-3´	5´-TTGGCCTTAGGGTTCAGGGGG - 3´

Reactions were initiated by adding a cofactor, namely, NADH, NADPH or a combination of the two cofactors (Sigma-Aldrich, St Louis, MO, USA), to a final concentration of 1.4 mmol/l. The reactions were performed at +37 ˚C for 40 min, and terminated by placing the reaction tubes into ethanol – dry ice bath. The steroids were extracted twice with 1 ml of diethyl ether (Merck Millipore, Merck KGaA,

Darmstadt, Germany), evaporated under nitrogen flow and reconstituted in 60 µl of acetonitrile-water (48:52, vol/vol). The converted substrate was analyzed by separating the [3H]-steroids using HPLC (PerkinElmer).

4.5 Steroid profiling (I, II)

The intratissue concentrations of E2, E1, T, DHT, P4 and Adione in the ovaries and adipose tissues of the WT and Hsd17b1-LacZ/Neo mice were analyzed per a validated gas chromatography tandem mass spectrometry method (Nilsson et al., 2015). Briefly, after addition of isotope-labeled standards, steroids were extracted in chlorobutane, purified on a silica column, and derivatized using pentafluorobenzylhydroxylamine hydrochloride followed by pentafluorobenzoyl chloride. Steroids were detected by Agilent 7000 triple quadrupole mass spectrometer using electron capture negative chemical ionization in multiple reaction monitoring mode and ammonia as the reagent gas. With this instrumentation, the limits of the quantification for serum were shown to be E2, E1, T, DHT, P4, and Adione were 0.5, 0.5, 8, 2.5, 74, and 12 pg/ml, respectively.

4.6 Histology and immunohistochemistry (I, II)

For the histological analyses, tissues were weighed and fixed with a 10 % buffered formalin solution for 24-48 hours in RT. Then, the tissue samples were paraffin-embedded and cut into 5-µm-thick sections. Afterwards, the sections were deparaffinized, rehydrated and stained either with hematoxylin-eosin (HE) or Periodic acid–Schiff (PAS).

For the immunohistochemical analyses, sections were preincubated at 60°C for 30 min and rehydrated. Antigen retrieval was performed in a pressure cooker for 20 min in 10 mmol/l citrate buffer (pH 6.0). Sections were then washed in PBS with 0.05 % Tween (PBS-T) followed by 30 min treatment in a humidified chamber at room temperature with PBS containing 10 % normal goat serum (NGS, Vector Laboratories Inc., Burlingame, CA, USA), 3 % BSA (Sigma-Aldrich, St. Louis, MO, USA) and 0.05 % Tween. After blocking, primary antibodies against CYP17A1 (dilution 1:2000, #PTGlab14447, Proteintech Group, Inc., Chicago, IL, USA), HSD3B1 (dilution 1:3000, kindly provided by Prof. Mason, The Queen's Medical Research Institute, University of Scotland, Scotland) or SF-1 (dilution 1:100, kindly provided by Prof. Morohashi, Kyushy University, Fukuoka, Japan) in PBS-T with 3 % BSA were applied, and the samples were incubated overnight in a humidified chamber at 4°C. Thereafter, samples were washed in PBS-T, and

endogenous peroxidase was blocked with 3 % H_2O_2 in PBS-T for 20 min at room temperature (RT). The samples were washed with PBS-T and blocked again against non-specific binding. Samples were then incubated with an anti-rabbit secondary antibody (HRP-labelled polymer against rabbit, Dako EnVision+ System, Dako North America Inc., Carpinteria, CA, USA) for 30 min at room temperature. Samples were washed again, and the positively stained cells were visualized with a 3,3' Diaminobenzidine (DAB) solution (Dako North America Inc., Carpinteria, CA, USA) according to the manufacturer's instructions. After visualization, samples were washed with water, counterstained with Mayer's hematoxylin, dehydrated and mounted.

To detect neutral lipids in the liver tissue sections, the tissue samples were dissected and frozen with isopropanol and dry ice. 10-µm-thick sections were cut, formalin-fixed, and washed with isopropanol and water. The sections were stained with Oil Red O (Sigma-Aldrich, St. Louis, MO, USA) and washed with isopropanol and water. Mounting was performed with Aquatex® (Merck, Darmstadt Germany).

4.7 Diet study (unpublished data)

The Hsd17b1-LacZ/Neo and WT male littermate mice were provided with normal diet (ND) or high fat diet (HFD) for 3 months, starting from 8 weeks of age. The Naglu-neo and WT male mice (n = 9-11) were fed only with ND, and weight gain was monitored weekly. Body composition, including the whole-body fat mass, lean mass and total water, was measured once every two weeks by quantitative nuclear magnetic resonance (NMR) scanning (EchoMRI-700, Echo Medical Systems, Houston, Texas, USA). At the beginning and end of the study, food consumption was measured in the home-cage environment.

4.8 Glucose tolerance test (GTT) (II)

GTTs for the Hsd17b1-LacZ/Neo and Naglu-Neo mice (n = 9-10) were performed at three months of age. Following four hours of fasting, the basal level of blood glucose was measured. Glucose (1 g/kg body weight) was injected intraperitoneally (i.p.) and the blood glucose concentration was measured with FreeStyle Lite (Abbot Diabetes Care Inc., USA) from a blood sample obtained from the saphenous vein 20, 40, 60 and 90 min after glucose injection.

4.9 Indirect calorimetry of Hsd17b1-LacZ/Neo mice (unpublished data)

Energy expenditure (EE), oxygen (O_2) consumption and respiratory exchange ratio (RER) were measured through evaluation of respiratory metabolism by indirect calorimetry (Oxylet system, Panlab SL, Barcelona, Spain). Measurements were carried out individually for each animal placed in chambers where they had free access to food and water. The first 2 hours were used for acclimation period. During this time the data was discarded and the monitoring period was for 22 hours. RER was calculated as the ratio of the volume of CO_2 produced by the volume of O_2 consumed. All the values were calculated with Metabolism v3.0 software (Panlab SL, Barcelona, Spain).

4.10 Expression of the genomic locus of *Naglu* and *Hsd17b1* genes (II)

To study whether the deletion of *Hsd17b1* gene affects *Naglu* expression *in vitro*, we built two expression constructs of this genomic region with and without the genomic fragment spanning the 1.8 kb coding region of *Hsd17b1*. The constructs were co-transfected with a luciferase-expressing reporter plasmid (pRL Renilla luciferase reporter vector, Promega, Madison, WI, USA) to COS cells using TurboFect kit (Thermo Fisher Scientific, Wilmington, DE, USA) according to the instructions provided by the manufacturer. Thereafter, the expression of *Naglu* in the transfected cells was analyzed by qRT-PCR and normalized to *luciferase* expression.

4.11 RNAseq analysis of the ovaries and white adipose tissue of Hsd17b1-LacZ/Neo mice (II)

The RNA was extracted and DNase-treated as mentioned above in chapter "4.3 Analysis of mRNA expression", and the sample quality was analyzed with Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The RNA from the ovaries and male gonadal fat was isolated from 6 WT controls and 6 Hsd17b1-LacZ/Neo mice. The samples were sequenced with the HiSeq2500 instrument (Illumina, San Diego, CA, USA), according to the manufacturer's instructions at the Finnish DNA Microarray and Sequencing Centre (Turku Centre for Biotechnology, the University of Turku) and single-end sequencing chemistry was used with 50 bp read length.

4.12 Glycosaminoglycan (GAG) measurement (II)

The glycosaminoglycan measurement was done according to a previously published method (Björnsson 1993). In brief, the snap-frozen samples were incubated in 0.9 % NaCl, 0.2 % Triton X-100 overnight at +4ºC in shaking, and then shortly homogenized with TissueLyser (Qiagen, Hilden, Germany) using a metal bead with a diameter of 7 mm. After homogenizing the tissues, the cell debris was centrifuged at 5000 g for 10 min at +4ºC and the supernatant was collected for glycosaminoglycan quantitation. Samples were mixed with 8 M guanidine-HCl and incubated for 15 min at RT. Solution containing 0.054 M sulfuric acid and 0.75 % Triton X-100 was applied to samples and mixed thoroughly. Alcian blue solution (Alcian Blue, 1.8 M sulfuric acid, 8 M guanidine-HCl and 10 % Triton X-100) was added and samples incubated for 1 hour at +4ºC. Samples were centrifuged at 12 000 g for 15 min and supernatant was discarded. The pellet was washed with 40 % DMSO, 0.05 M $MgCl_2$ solution and then mixed with dissociation mixture (4.0 M guanidine-HCl, 33 % isopropanol) and agitated at RT for 15 min. The absorbance of samples was determined at 600 nm.

4.13 Statistical analyses (I, II)

Statistical analyses were conducted using SigmaPlot/Stat program (Systat Software GmbH, Erkrath, Germany) or GraphPad Prism 7 software (GraphPad Software, Inc., La Jolla, CA, USA). Statistical analyses included unpaired t-test, Student's t-test, one way ANOVA, two way repeated measure ANOVA or Mann-Whitney test. One way or two way ANOVA was followed with Bonferroni post hoc test. Significance was determined as $P<0.05$, and standard deviation (SD) or standard error of the mean (SEM) was used in visualizations along with mean values.

5 RESULTS

5.1 Establishment of the genetically modified mouse lines used in the thesis (I-II)

To study the physiological function of the HSD17B1 enzyme, we generated a mouse model, Hsd17b1-LaZ/Neo, with the *Hsd17b1* gene deletion. The targeting method replaced all 6 coding exons of the *Hsd17b1* gene with the β-galactosidase (*lacZ*) and neomycin (*neo*) cDNAs, which were inserted in the translation start codon of the *Hsd17b1* locus (I: Fig. 1A and II: Fig. 1A and 1B). Analysis of *Hsd17b1* mRNA expression in the C57Bl/6N wild type (WT) mice was performed by qRT-PCR and as expected, the expression levels of *Hsd17b1* mRNA were highest in the ovaries. Furthermore, we detected *Hsd17b1* expression in the adult testis and white adipose tissue, but *Hsd17b1* expression in these tissues was much lower than in the ovary (I: Fig. 1B). With qRT-PCR, we confirmed that the ovaries of the Hsd17b1-LaZ/Neo mice did not have *Hsd17b1* mRNA expression (I: Fig. 1C). Moreover, the whole-mount X-gal stains of the heterozygous (Hez) and homozygous (Hoz) Hsd17b1-LacZ/Neo ovaries established the expression of the *lacZ* reporter gene in the ovarian follicles, a site of endogenous *Hsd17b1* gene expression (I: Fig. 1D).

Due to the potential effect of the promoter presence as part of the inserted *neo* cassette, we deleted the *neo* expression cassette from the targeted *Hsd17b1* locus by crossing the Hsd17b1-LacZ/Neo mice with those expressing *Cre* recombinase under the Rosa26-promoter. The appropriate Cre recombination event in the Hsd17b1-LacZ mice was detected by PCR analysis to ensure that the *neo* cassette was removed in the germ line, resulting in a lack of the selection cassette in all cells (II: Fig. 1C). Furthermore, to overexpress the human enzyme in our null mouse model, we crossed the Hsd17b1-LacZ/Neo mice with the HSD17B1TG mice expressing human *HSD17B1* in all tissues under the chicken beta-actin promoter (II: Fig. 1D). In addition, we studied fertility, adiposity, and *Hsd17b1* expression in the mice with the disrupted *Naglu* gene generated by an insertion of a *neo* cassette within exon 6 (Naglu-Neo mice).

5.2 HSD17B1 enzyme in female reproduction (I)

5.2.1 Deleting the Hsd17b1 genomic region altered the steroid balance in the ovary

Mouse HSD17B1 is an enzyme that converts E1 to E2 and Adione to T. Accordingly, when measured in the ovarian homogenates of the Hsd17b1-LacZ/Neo mice, the conversion of E1 to E2 was significantly reduced in the proestrus and estrus samples compared with the controls. The activity measurements were carried out in the presence of NAD(P)(H), NAD(H), or both, and the results indicated that HSD17B1 contributed to the capacity of the total ovarian HSD17B activity towards converting E1 to E2 *in vitro* by 75 to 95 % (I: Fig. 4A). When we performed the qRT-PCR analysis, we could not detect any compensatory effect of HSD17B12, as the expression level of *Hsd17b12* was not altered. However, *Hsd17b7* expression in the ovaries of the Hsd171b-LacZ/Neo mice was fairly upregulated in the proestrus samples (I: Fig. 5A), indicating that HSD17B7 could be responsible for the E1 to E2 conversion still observed in the Hsd17b1-LacZ/Neo ovarian samples (< 25 % of the WT activity) (I: Fig. 4A). The physiological role of the HSD17B7 enzyme in the ovaries has been difficult to study, as the full HSD17B7KO mice die *in utero* due to imbalanced cholesterol metabolism (Jokela et al., 2010). However, as presented in Figure 8, the *Hsd17b7* gene expression is unique in the mouse ovaries compared with other cholesterol biosynthesis enzyme-coding genes, and expression is particularly high in the ovaries of pregnant mice.

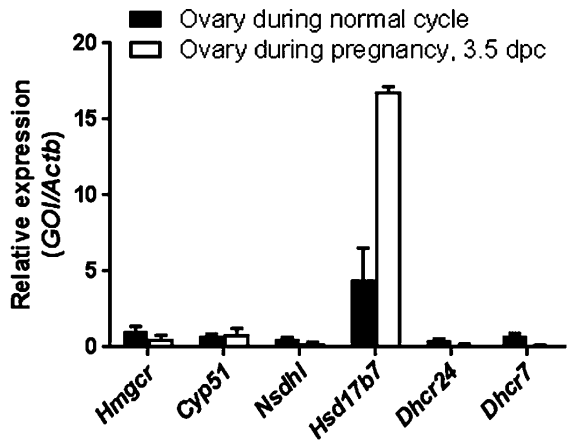

Figure 8. Quantitative RT-PCR analyses of the cholesterogenic genes in the ovaries. *Hmgcr*, *Cyp51*, *Nsdhl*, *Hsd17b7*, *Dhcr24* and *Dhcr7* expressions in the ovaries during normal cycling (black bars) and during pregnancy at 3.5 dpc (white bars) of adult wild type mice (n=6). The results were normalized to *Actb* expression. GOI = gene of interest.

To further characterize the ovarian steroid synthesis, the intraovarian steroid concentrations were measured by gas chromatography/tandem mass spectrometry. Concomitantly, with the reduction in HSD17B activity, a substantial increase in the E1 to E2 ratio was evident in the ovaries during proestrus, estrus and pseudopregnancy (I: Fig. 4B). In addition, the Adione to T ratios were higher in the Hsd17b1-LacZ/Neo mice than in the control ovaries during the different phases of the estrous cycle (7-fold at proestrus). Surprisingly, the strongest effect was observed for the Adione to T ratio in the ovaries of the pseudopregnant Hsd17b1-LacZ/Neo mice (38-fold, $P < 0.01$; I: Fig. 4C). On the other hand, the absolute concentrations of these sex steroids were not significantly different between the control and Hsd17b1-LacZ/Neo ovaries.

Progesterone (P4) is one of the hormones produced by luteal cells and regulates female fertility. We detected a clear reduction in the P4 concentration in the ovaries of mice with disrupted *Hsd17b1* gene at estrus and during pseudopregnancy (3-6-fold, depending on the hormonal status; I: Fig. 4D). Gonadotropins were also analyzed, and no changes were observed in the FSH levels, whereas LH was elevated 5-fold in Hsd17b1-LacZ/Neo mice at the estrus stage compared with the littermate controls.

5.2.2 Lack of HSD17B1 enzyme activity did not have an effect on the pubertal onset or reproductive cycle

While some of the HSD17B enzymes have been shown to be essential for embryonic development (Jokela et al., 2010; Rantakari et al., 2010), a full deletion of the *Hsd17b1* coding region did not have any effect on the genotype ratios of the pups born from the heterozygous (HEZ) breedings. By breeding the HEZ male and female mice, a total of 26 % of the pups born were genotyped as wild type (WT), 46 % as HEZ, and 28 % as homozygous (HOZ). When determining vaginal opening in the Hsd17b1-LacZ/Neo and littermate controls, it became evident that deleting this steroidogenic enzyme did not have a significant effect on female pubertal development. The day of vaginal opening did not differ significantly between the WT (d 30 ± 0.8) and Hsd17b1-LacZ/Neo (d 33 ± 3.4) mice. Moreover, the macroscopic appearance of all reproductive organs were normal compared with the WT littermates. However, the weight of the ovaries was increased in the Hsd17b1-LacZ/Neo mice during estrus and pseudopregnancy. Normally, E2 regulates the priming of the uterus in mice, causing an increase in the wet weight. Although the E2 level was not changed in the ovaries of the Hsd17b1-LacZ/Neo mice, the uterine weight of the mice did not follow the typical cycle-dependent change: The proestrus uterine wet weight of the WT controls was higher than the estrus uterine

wet weight of the WT controls, but this effect was not observed in the Hsd17b1-LacZ/Neo mice.

To test the reproductive performance further, the estrous cycle was followed for 21 days with vaginal smear samples. The estrous cycle was shown to be normal in the Hsd17b1-LacZ/Neo mice; the length in the Hsd17b1-LacZ/Neo females was 6 d ± 2, and the lenght in the WT females was 5 d ± 1. In addition, the time spent in different estrous cycles did not differ between the Hsd17b1-LacZ/Neo and control mice (I: Fig. 2D).

5.2.3 HSD17B1 is needed for pregnancy and pseudopregnancy

As the Hsd17b1-LacZ/Neo females had normal pubertal onsets and normal estrous cycles, we examined the fertility in the female mice further. First, we performed an ovulation test, where the Hsd17b1-LacZ/Neo females were also found to ovulate normally (6.4 ± 0.8 oocytes/ovulation after mating). However, when the Hsd17b1-LacZ/Neo females were bred with male mice of known fertility, we identified a severe problem in the fertility of the Hsd17b1-LacZ/Neo females. After 10 WT and 10 Hsd17b1-LacZ/Neo female mice were mated with WT males for 60 d, the WT female littermates produced 23 litters with 232 pups altogether, while the mutant females produced only 6 litters with 32 pups. Within 60 days of breeding, the observed number of plugs was 56 in the Hsd17b1-LacZ/Neo mice and 32 in the WT mice, indicating that the mating of the Hsd17b1-LacZ/Neo females seldom resulted in successful pregnancies with born offspring. In addition, the litter size of the Hsd17b1-LacZ/Neo females was significantly smaller than that of the WT females (WT = 10.1, Hsd17b1-LacZ/Neo = 6.4, $P < 0.01$) (I: Table 1). To test whether the fertility defect detected in the Hsd17b1-LacZ/Neo mice could also be related to problems in initiation of pseudopregnancy, we mated 7 Hsd17b1-LacZ/Neo and 5 control females with infertile male mice. Vaginal plugs were then checked daily for 30 days. The plugs were observed regularly (at an interval of 3-7 days) in the Hsd17b1-LacZ/Neo mice, while in the WT females, the plugs were presented after a 10- to 12-long pseudopregnancy period (I: Fig. 3). With this test, it became evident that pseudopregnancy was not maintained properly in the Hsd17b1-LacZ/Neo mice.

5.2.4 Hsd17b1 absence resulted in a luteinization defect

The ovary is a complex endocrine organ that consists of multiple cellular structures, such as ovarian follicles at different developmental stages, the CL and ovarian stroma. When the histology of 3-month-old females at proestrus and estrus was analyzed, our analysis revealed that of follicles from different stages of folliculogenesis were present in the Hsd17b1-LacZ/Neo ovaries, as was the CL. In addition, the number of follicles at the different developmental stages was not changed in the proestrus samples. Nevertheless, the histological structure of the basement membrane of the CL of the Hsd17b1-LacZ/Neo mice was less defined than that of the WT CL (I: Fig. 6).

We mated the Hsd17b1-LacZ/Neo females with infertile males and collected the ovaries at 3.5 days *post coitum* to evaluate the CL more closely. The number of CLs was significantly lower in the ovaries of the pseudopregnant mice lacking HSD17B1 activity than in the ovaries of the WT mice (I: Fig. 6G). In line with the histological observations, the mRNA expression levels of markers for CL function (*Hsd17b7*, *Cyp11a1* and *Lhcgr*) were downregulated significantly. Notably, expression of *Hsd17b7* disappeared completely from the ovaries of pseudopregnant Hsd17b1-LacZ/Neo females. In contrast, the most marked expression change in the group of steroidogenic genes was with the expression of *Cyp17a1*, whose level was 12-fold higher in the ovaries of the pseudopregnant Hsd17b1-LacZ/Neo mice than in the ovaries of the WT mice (I: Fig. 5). We also observed by immunohistological staining that the upregulated expression of *Cyp17a1* was specifically localized to the follicular theca cells (I: Fig. 7C).

Interestingly, large granular cell bundles were also observed in the stroma of the Hsd17b1-LacZ/Neo ovaries and uteri, regardless of the estrous cycle or pseudopregnancy. We then tested the steroidogenic activity of these cells with immunohistochemistry of SF1, HSD3B2 and CYP17A1 and found that the cells were negative for these markers of steroidogenesis (I: Fig. 7A and B). Additionally, we tested the cells for neutral lipids with Oil Red O staining and found that the cell bundles were negative for lipid accumulation (data not shown).

5.3 Metabolic phenotype of the Hsd17b1-LacZ/Neo mice (II and unpublished data)

5.3.1 Body composition of the Hsd17b1-LacZ/Neo mice was altered

Both sexes of the Hsd17b1-LacZ/Neo mice had decreased fat masses when they became older. This was first discovered in 9 month-old females with smaller body weights. Further studies showed a decrease in fat mass in males at 3 months of age, which was many months before the decrease became detectable in the body weight. This led us to perform a diet study, where adult (8 weeks of age) mice were fed with ND or HFD for 3 months and where the body weights of the mice were followed. No difference in the body weight between the genotypes was detected with either the ND or HFD diet. However, there was a great reduction in fat mass in the Hsd17b1-LacZ/Neo mice per the body composition measurements, and we further discovered that the HFD did not have effects on the body weight or adiposity of the Hsd17b1-LacZ/Neo mice. In these mice, there was a substantial reduction in the weights of all white adipose fat pads and even in the interscapular BAT. Together with the reduced fat mass, the Hsd17b1-LacZ/Neo mice had significantly increased lean mass (II: Fig. 5B).

5.3.2 Small adipocytes and browning of the white adipose tissue were observed in the Hsd17b1-LacZ/Neo mice

Histological analysis demonstrated smaller adipocytes in the WAT of the Hsd17b1-LacZ/Neo mice than in the WT littermates, and the sizes of the adipocytes did not increase when the Hsd17b1-LacZ/Neo mice were fed with the HFD for three months. Histological analysis revealed browning of the WAT, and this observation was supported by the upregulation of *Ucp1* in the WAT (a marker for BAT, data not shown). The tissue weight of the BAT at the time of sacrifice was decreased in the Hsd17b1-LacZ/Neo mice, and in line with this, the lipid droplet size in the BAT was revealed to be smaller by histological analysis (II: Fig. 5E). Furthermore, the HFD did not enlarge the lipid droplet size in the WAT of Hsd17b1-LacZ/Neo mice as it did in the WT mice (Figure 9).

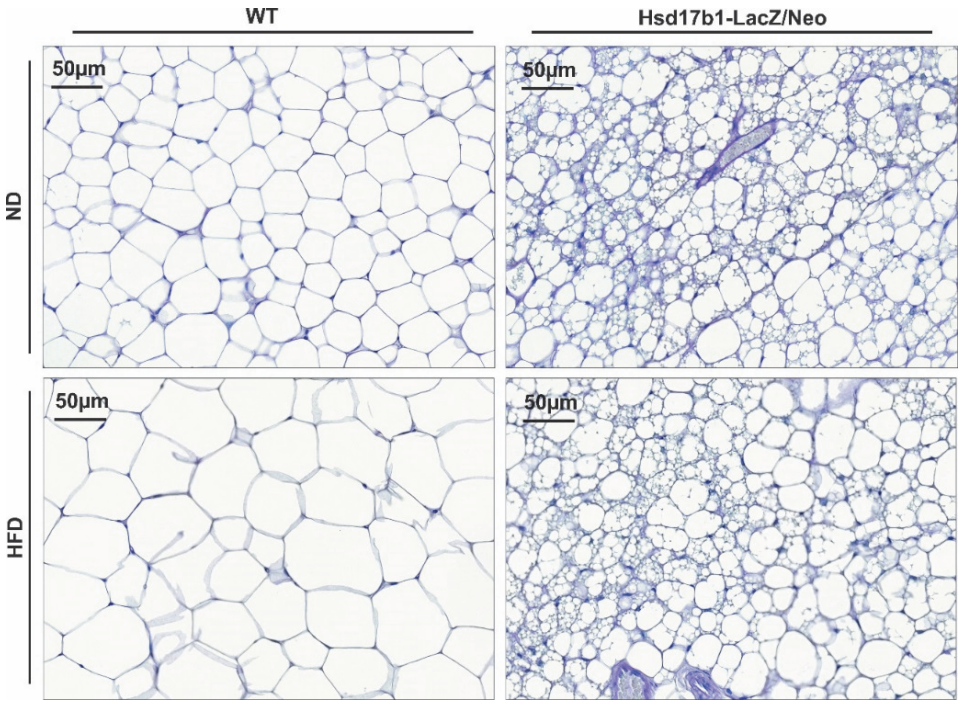

Figure 9. Adiposity of the Hsd17b1-LacZ/Neo male mice. Histological analysis of the subcutaneous white adipose tissue (scWAT) revealed smaller adipocytes and browning of adipose tissue in the Hsd17b1-LacZ/Neo mice compared to the WT littermates.

5.3.3 Glucose tolerance was improved in the Hsd17b1-LacZ/Neo mice, while lipids accumulated in the liver

The weight of the liver was significantly increased in the Hsd17b1-LacZ/Neo mice compared with the WT littermates. Furthermore, the histological analysis revealed lipid accumulation in the livers of the Hsd17b1-LacZ/Neo mice. In these mice, liver steatosis was detected on the normal diet, and HFD further increased fat accumulation in the hepatocytes, evidenced by hematoxylin-eosin (HE) staining and Oil Red O staining (II: Fig. 6A-D and data not shown). Despite the liver steatosis, the glucose tolerance of the mice improved under the HFD and the fasting glucose was low in both the control-diet (ND) and HFD cohorts at the time of sacrifice. In addition, the fasting serum insulin levels of the Hsd17b1-LacZ/Neo mice were decreased, suggesting that the improvement in glucose homeostasis was due to improved insulin sensitivity (Figure 10).

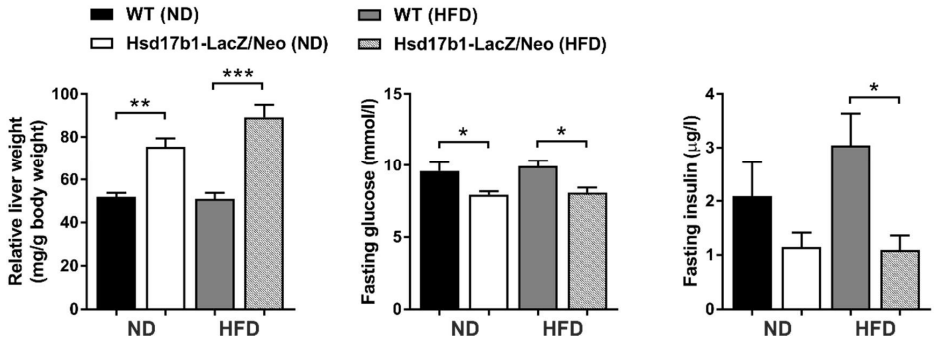

Figure 10. Liver weight, fasting serum glucose and insulin concentration of Hsd17b1-LacZ/Neo males. The liver weight was increased significantly in the Hsd17b1-LacZ/Neo mice on both the ND and HFD (5 months old, n = 9-10). Fasting glucose was decreased, as were the insulin levels when compared Hsd17b1-LacZ/Neo and WT mice on ND or HFD. The column indicates the mean values, and the error bar indicates the standard error of mean (SEM). *P < 0.05, **P < 0.01 and ***P < 0.001. The statistical comparison was performed only inside the ND and HFD groups with one way ANOVA followed by Bonferroni's comparison test.

5.3.4 Decreased lipolysis and downregulation of lipogenesis in the liver

Fat accumulation is determined by the balance between fat synthesis (lipogenesis) and fat breakdown (lipolysis/fatty acid oxidation). Although lipids accumulated in the livers of the Hsd17b1-LacZ/Neo mice, *de novo* lipogenesis was reduced, according to the mRNA levels of *Fasn* and *Acaca.* Lipolysis, on the other hand, was reduced; the fasting triglyceride level was increased, and the non-esterified free fatty acid (NEFA) and glycerol levels were decreased in the serum (Figure 11).

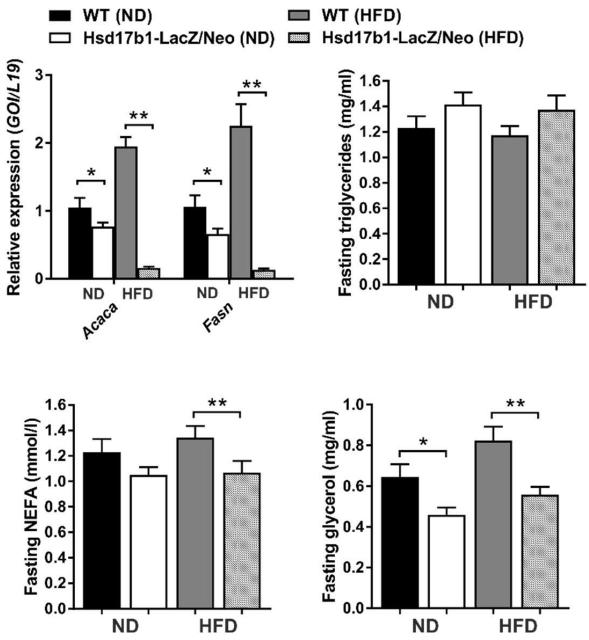

Figure 11. Balance between lipogenesis and lipolysis in the Hsd17b1-LacZ/Neo mice. *Acaca* and *Fasn* were significantly downregulated in the liver (n = 6), especially in mice on the HFD, indicating reduced *de novo* lipogenesis. Lipolysis was decreased, as the serum NEFA and glycerol levels were reduced and as the triglycerides were increased (n=9-10). The column indicates the mean value, and the error bar indicates standard error of mean (SEM). *P < 0.05, **P < 0.01 and ***P < 0.001. The statistical comparison performed with t-test inside the ND and HFD groups.

5.3.5 Energy homeostasis was not significantly altered in the Hsd17b1-LacZ/Neo mice

To test the energy balance in the Hsd17b1-LacZ/Neo mice, we first examined energy intake in the home cages and metabolic cages. The energy intake was not different between the WT and Hsd17b1-LacZ/Neo mice on the ND or HFD. Urine was collected in the metabolic cages, and we detected a decreased amount of urination by Hsd17b1-LacZ/Neo mice. Oxygen consumption (VO_2), energy expenditure (EE) and the respiratory exchange ratio (RER) were measured with indirect calorimetry, and all parameters were normal and similar between Hsd17b1-LacZ/Neo and WT mice (data not shown).

5.3.6 Steroid ratios in white adipose tissue

The intratissue steroid profiles were prepared from the gonadal fat of 3 months old male and female mice to elucidate the role of the HSD17B1 enzyme in white adipose tissue. In the males, the E2 levels were not high enough to measure reliably, but in the females we discovered a 50 % reduction from the normal level measured in the WT females, although the difference did not reach significance. In contrast to E2, E1 was increased slightly in the Hsd17b1-LacZ/Neo female adipose tissue. Thus, the E1/E2 ratio was significantly higher in the adipose tissue of the Hsd17b1-LacZ/Neo females than in the WT females ($P < 0.05$), which was in line with the expected activity of HSD17B1 for converting E1 to E2. Furthermore, the Adione to T ratio was also significantly higher in the females ($P < 0.01$) but not in the males. These results (Table 3) show that HSD17B1 activity has the potential affect steroid synthesis in white adipose tissue.

Table 3. White adipose tissue steroid concentrations of 3-month-old Hsd17b1-LacZ/Neo females and males. Values are presented as mean of pg/g of the tissue ± SEM of ≥ 5 animals. Statistical analysis performed with unpaired t-test. *P < 0.05 and **P < 0.01.

		E1	*E2*	*E1/E2*	*Adione*	*T*	*Adione/T*
♀	**WT**	416.1 ± 94.04	49.77 ± 11.31	11.53 ± 4.399	265 ± 57.84	34.23 ± 7.374	8.478 ± 1.578
	Hsd17b1-LacZ/Neo	879.2 ± 240.5	26.86 ± 6.696	34.64 ± 7.35 *	877.5 ± 220 *	33.91 ± 5.904	19.94 ± 3.24 **
♂	**WT**	2513 ± 1551	NM	NM	4835 ± 2362	32298 ± 25264	0.837 ± 0.3866
	Hsd17b1-LacZ/Neo	677.8 ± 251.4	NM	NM	7651 ± 2217	55211 ± 24134	0.3848 ± 0.0968

5.4 Deletion of the *Hsd17b1* region resulted in downregulation of a neighboring gene, *Naglu* (II)

Global gene expression profiling was performed with RNA sequencing to assess the transcriptional consequences of HSD17B1 loss in the ovaries (diestrus and pseudopregnant) and in male gonadal white adipose tissue (gWAT). In the white

adipose tissue, the expression levels of 38 genes were upregulated, the levels of 105 genes were downregulated in the absence of *Hsd17b1*, and some of the fold changes (FCs) observed for the differentially expressed genes were relatively high (FC -40.5 to +143.9). Among these genes, a severe reduction in N-acetyl-alpha-glucosaminidase (*Naglu*) mRNA was observed with a FC of -40.5. Similar effects were observed in the ovaries of pseudopregnant (FC -29.2) and diestrus (FC -20.3) mice. This gene, which codes for the lysosomal enzyme NAGLU, is located immediately (739 bp) upstream of the *Hsd17b1* transcriptional start site. To confirm that this downregulation of *Naglu* was not due to error in gene targeting, we confirmed that the targeting event was properly carried out by sequencing the genomic region in the Hsd17b1-LacZ/Neo mice from the beginning of exon 6 to the beginning of the targeting cassette.

5.4.1 Overlap of mouse Hsd17b1 gene with an enhancer region regulating Naglu gene expression

After analyzing the RNA sequencing results, we confirmed the expression change in *Naglu* by qRT-PCR. The data indicated that in the Hsd17b1-LacZ/Neo mice, the expression of *Naglu* was severely downregulated in all tissues analyzed (liver, kidney, adrenal gland, gonadal WAT and BAT, testis, epididymis, ovary and uterus). mRNA expression of *Naglu* was present in nearly all tissues with variable quantities, and downregulation of gene expression in the tissues varied between 7- to 119- fold.

Due to the possibility that the additional promoter driving the neo-resistance gene in the targeting cassette could interfere with the expression of neighboring genes, we deleted the *neo* cassette from the targeted *Hsd17b1* locus by crossing the Hsd17b1-LacZ/Neo mice with mice expressing Cre recombinase under the Rosa26 promoter. The crossing generated mice (Hsd17b1-LacZ) with the *neo* cassette removed in the germ line and a consequent lack of the selection cassette from all cells. However, in these mice, the expression of *Naglu* was still downregulated in a similar manner to the mice that had the *neo* cassette insertion. Therefore, the existence of the *neo* cassette in the targeted *Hsd17b1* locus was not causing downregulation of *Naglu*.

To test whether *Naglu* expression was dependent on HSD17B1 enzyme activity or whether the dysregulation was entirely dependent on the altered genomic structure, we generated mice that have *Hsd17b1* gene deletion, but express human *HSD17B1* ubiquitously, by crossing the Hsd17b1-LacZ/Neo females with the HSD17B1TG males. *Naglu* expression remained low in the Hsd17b1-LacZ/Neo X HSD17B1TG mice, indicating that restoring HSD17B1 enzyme activity to the Hsd17b1-

LacZ/Neo genetic background did not have any effect on the transcription of *Naglu*. Furthermore, we tested the interaction between the *Hsd17b1* and *Naglu* genes with two BAC constructs *in vitro*: One covered the 5-kb genomic region beginning upstream from the *Naglu* transcriptional start site to the 3' region of the *Hsd17b1* gene, and the other was a similar construct that lacked a 2.1-kb-long fragment of the *Hsd17b1* gene. By transfecting these constructs together with luciferase into COS cells, it became evident that the deletion of the *Hsd17b1* gene in BAC reduced the expression levels of the *Naglu* gene similarly to those observed in the Hsd17b1-LacZ/Neo mice *in vivo*.

In addition, we analyzed the expression of *Hsd17b1* in the Naglu-Neo mice with the disrupted *Naglu* gene generated by an insertion of a targeting cassette within exon 6. The qRT-PCR data showed that this insertion did not affect the expression of *Hsd17b1*. In conclusion, both the *in vivo* and *in vitro* data demonstrate that the genomic region of the *Hsd17b1* gene includes an essential regulatory element for the *Naglu* gene, while disruption of the last exon of *Naglu* does not interfere with *Hsd17b1* expression.

5.4.2 Downregulation of Naglu in mice with disrupted Hsd17b1 gene resulted in accumulation of glycosaminoglycans

The NAGLU enzyme is located in lysosomes, where the enzyme is involved in the stepwise breakdown of a glycosaminoglycan (GAG) known as heparan sulfate. A deficiency in this enzyme results in the accumulation of GAGs in lysosomes. Accordingly, an increased level of GAGs was found in most of the tissues analyzed from the mice with the disrupted *Hsd17b1* gene. The highest concentrations were measured in the liver and kidney, consistent with the observation in the Naglu-Neo mice. Significant accumulation was also observed in the ovary and uterus of the Hsd17b1-LacZ/Neo mice, where we previously detected large vacuole-like structures in the stroma. Interestingly, in the testis, where the highest *Naglu* expression level was observed in the WT mice, the remaining 10 % of the expression present in the Hsd17b1-LacZ/Neo mice was enough to avoid the accumulation of GAGs. Together with the accumulation of GAGs in different tissues, the cells were stained with antibodies against lysosomal markers, LAMP1 and LIMP2. High intensity staining was observed especially in the kidney, epididymis, ovary and uterus of the Hsd17b1-LacZ/Neo mice. Moderate staining was observed in the liver. In the testis, there was no difference between the WT and Hsd17b1-LacZ/Neo mice. The foam cells in the stroma of the ovary and uterus were positive for the lysosomal

markers, confirming that the vacuolated phenotype of the female reproductive tissue comprised the accumulation of partially degraded glycosaminoglycans when an appropriate level of the NAGLU enzyme was lacking.

5.4.3 Metabolic phenotype of the Hsd17b1-LacZ/Neo mice was a result of reduced expression of Naglu

To elucidate whether the decreased adiposity in the Hsd17b1-LacZ/Neo mice was due to either the disrupted HSD17B1 or disrupted NAGLU activity, we analyzed the body weights and compositions of the Hsd17b1-LacZ/Neo X HSD17B1TG and Naglu-Neo males in the same manner as performed for the Hsd17b1-LacZ/Neo mice. The weights of the mice were followed for three months starting from eight weeks of age, and no body weight difference was detected in the Naglu-Neo male mice. However, there was a great reduction in fat mass and a significant increase in lean mass detected in the Naglu-Neo mice, an observation that was also made in the Hsd17b1-LacZ/Neo and Hsd17b1-LacZ/Neo X HSD17B1TG mice. As observed in the Hsd17b1-LacZ/Neo mice, a substantial reduction in all of the white adipose fat pads and in the interscapular BAT was evident in the Naglu-Neo mice. Detailed histological examination of the subcutaneous (scWAT) and gonadal WAT (gWAT) revealed a smaller adipocyte size in Naglu-Neo males compared with WT littermates, and browning was detected in the scWAT of the Naglu-Neo mice. The liver weight was significantly increased, and furthermore, histological analysis revealed a partial lipid accumulation in 20 % of the mice. Regardless of the lipid accumulation in the liver, the glucose tolerance was improved in the Naglu-Neo mice. Thus, all metabolic phenotypes observed in the mice with *Hsd17b1* gene deletion could be replicated in the Naglu-Neo mice, strongly suggesting that the metabolic phenotype of the *Hsd17b1*-deficient mice was due to severe downregulation of *Naglu*.

5.4.4 Subfertility and steroid alterations are caused by the absence of the HSD17B1 enzyme

To confirm that our results for female fertility were caused by disruption of the targeted *Hsd17b1* gene and not the off-target effects of genetic manipulation, we followed the estrous cycle and performed fertility tests on 2-4-month-old Naglu-Neo females. The estrous cycle appeared normal, as observed for the Hsd17b1-LacZ/Neo mice. Furthermore, the time spent in the different phases of the estrous cycle during a 21-d follow-up period was similar in the Naglu-Neo mice and in the WT littermates. However, the mRNA expression of genes that altered in the

Hsd17b1-LacZ/Neo mice compared with WT littermates (*Hsd17b1*, *Hsd17b7*, *Cyp17a1* and *Lhcgr*) was not change significantly in the ovaries of pseudopregnant Naglu-Neo mice. In contrast, the expression of *Hsd17b7* in the ovaries was upregulated upon depletion of *Naglu* rather than downregulated, as observed in the ovaries of the pseudopregnant Hsd17b1-LacZ/Neo mice. Additionally, immunohistochemical staining and qRT-PCR analysis confirmed that CYP17A1 was not upregulated in the theca cells of the Naglu-Neo females. The histological and electron microscopy analyses demonstrated lysosomal accumulation in the stroma of the ovaries (Figure 12). When female Naglu-Neo and control littermate mice were mated with fertile WT male mice for 60-d, it became evident that the female mice presented with normal fertility and litter sizes. These results demonstrate that subfertility in the Hsd17b1-LacZ/Neo mice is caused by the loss of HSD17B1 activity and not by the loss of NAGLU activity.

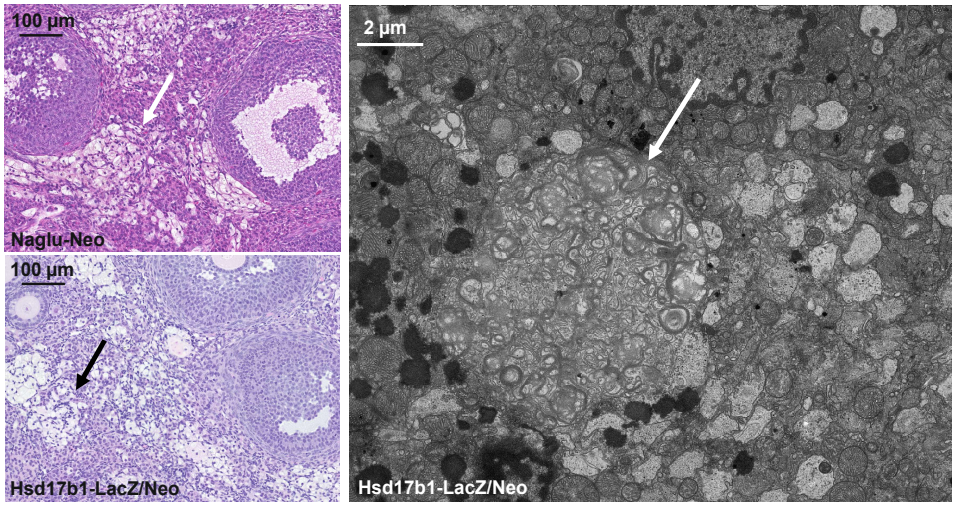

Figure 12. Histology and electron microscopy (EM) pictures of the stroma of 3-month-old Naglu-Neo and Hsd17b1-LacZ/Neo ovaries. Lysosomal hypertrophy (arrows) was similar in the Naglu-Neo and Hsd17b1-LacZ/Neo ovaries.

6 DISCUSSION

We set out to study the role of the HSD17B1 enzyme in female fertility and in the regulation of metabolism, especially in adipose tissue metabolism. The questions were approached by generating a knockout mouse model (Hsd17b1-LacZ/Neo) by replacing the whole coding region of the *Hsd17b1* gene with a *lacZ* reporter gene and neomycin resistance (*neo*) gene insertion. The mRNA expression of *Hsd17b1* was verified to be absent in the Hsd17b1-LacZ/Neo mice, showing that the *Hsd17b1* gene was efficiently deleted from the mice. As the inserted construct contained a gene coding for β-galactosidase, we confirmed that the heterozygous and homozygous ovaries showed X-gal staining in the growing follicles, a site for the endogenous expression of *Hsd17b1* (Akinola et al., 1997; Pelletier et al., 2004). Thus, deletion of the *Hsd17b1* gene was successfully established for the present study. However, during the metabolic characterization of the knockout mice, it became evident that the gene targeting method used for deleting *Hsd17b1* had disrupted the transcription of a neighboring gene coding for NAGLU and consequently caused a severe downregulation of *Naglu* in all tissues studied. The downregulation was also observed in the ovaries and white adipose tissue, thus confounding the interpretation of the results obtained with the Hsd17b1-LacZ/Neo mice.

6.1 HSD17B1 enzyme activity was needed for the initiation or maintenance of pregnancy and pseudopregnancy

Granulosa cells are somatic cells that closely surround the developing oocyte, and these cells are responsible for the production of steroid hormone, especially E2 (Mason et al., 1994). E2 synthesis involves collaboration between the theca cells surrounding the follicle, which produce androgens in response to LH. Furthermore, androgens then diffuse into granulosa cells, where the androgens are converted to estrogens by aromatase (CYP19A1) and where E1 is reduced to E2 by HSD17B1 (McNatty et al., 1979; Strauss et al., 2014). Due to the specific expression of mouse *Hsd17b1* in granulosa cells, the HSD17B1 enzyme is expected to be the enzyme responsible for gonadal E2 production in female mice. Moreover, the human *HSD17B1* gene is expressed in steroidogenic organs, granulosa cells and the placenta, which makes the present study exceptionally interesting from a translational point of view (Fournet-Dulguerov et al., 1987; Ghersevich et al., 1994b; Zhu et al., 2002). However, the actual whole-body function of the HSD17B1 enzyme has remained unsolved, as most of the functional studies have been previously performed only *in vitro*.

The significance of the HSD17B1 enzyme in estradiol production was confirmed by the present study carried out with the Hsd17b1-LacZ/Neo mouse model. The HSD17B activity analysis from the tissue homogenates of the knockout ovaries demonstrated that *Hsd17b1* deletion resulted in a 75 to 95 % reduction in the total HSD17B activity towards converting E1 to E2. This was the first *in vivo* evidence of the importance of the HSD17B1 enzyme in ovarian estradiol production, which was a result for the HSD17B1 enzyme that has been expected for several decades according to *in vitro* studies. The significant reduction in HSD17B activity indicates that the other HSD17Bs involved in the E1 to E2 conversion (HSD17B7 and HSD17B12) are not capable of compensating for the lack of HSD17B1 (Luu-The et al., 2006; Nokelainen et al., 1998). Therefore, it was surprising that the intraovarian steroid concentrations of E1, E2, Adione, or T in the Hsd17b1-LacZ/Neo female mice were not significantly altered when compared with the WT littermates. However, the relative concentrations of the 17-ketosteroids (Adione, E1) and the 17beta-hydroxysteroids (T and E2) were increased in the *Hsd17b1* knockout ovaries, demonstrating a role for HSD17B1 in the synthesis of highly active 17beta-hydroxysteroids.

The estrogens produced by granulosa cells have a negative feedback effect on androgen production in theca cells. This is mediated via estrogen receptor 1 (ESR1), as estrogens bind to ESR1 through which they it suppress *Cyp17a1* expression and subsequently reduce androgen synthesis (Couse et al., 2003). In the Hsd17b1-LacZ/Neo females, *Cyp17a1* expression was most markedly upregulated among the steroidogenic enzyme genes. The observed induction of CYP17A1 in the Hsd17b1-LacZ/Neo ovaries was localized by immunohistochemistry to the theca cells, and similar upregulation has been observed previously in ESR1 KO mice (Taniguchi et al., 2007) indicating a lack of estrogen signaling in the theca cells. This indicates that the intracrine actions of sex steroids (Labrie, 2015) in the ovary and the communication between the theca and granulosa cells is disrupted in the *Hsd17b1*-deficient mice.

In a normal situation, the expression of *Hsd17b1* is induced during pubertal maturation and estrogens begin to be produced by granulosa cells. To our surprise, even though the sex-steroid balance and local steroid synthesis were imbalanced in the Hsd17b1-LacZ/Neo females, the knockout females established their vaginal opening around the same age as the WT littermates. Vaginal opening is the first sign of puberty in females, and although vaginal opening is an apoptotic event, it is initiated by E2 (Levine, 2015). In addition, the estrous cycle functioned normally in the Hsd17b1-LacZ/Neo females when followed for 21 days. These observations of the female reproductive phenotype led to the conclusion that E2 production was not markedly disrupted. Thus, it is likely that HSD17B activity is not a rate-limit-

ing step in the production of appropriate levels of circulating E2 and that the severely reduced HSD17B activity identified in the knockout ovaries is sufficient for proper E2 production to support estrogen effects in peripheral tissues.

Although the Hsd17b1-LacZ/Neo mice presented with normal pubertal onset, a normal estrous cycle and an ability to ovulate, fertility was severely impaired because of ovarian failure. The *Hsd17b1* knockout females needed substantially more matings to conceive, and the litter size was greatly reduced. This indicates that the subfertility in the Hsd17b1-LacZ/Neo mice was caused by disrupted implantation or an inability to maintain pregnancy. In addition to P4, careful regulation of E2 production and action is one of the important factors for the uterine receptivity during implantation (Ma et al., 2003). Hence, proper estrogen signaling for successful implantation in the Hsd17b1-LacZ/Neo mice has been disrupted. Alternatively, in the Hsd17b1-LacZ/Neo mice, we observed a defect in the CL, which is known to produce high levels of P4 for fetal survival during pregnancy. The *Hsd17b1* knockout females had morphologic changes in their luteal cells and a reduced number of histologically defined CLs, especially during pseudopregnancy. The initiation of pseudopregnancy was weakened, further indicating an inadequate CL function. Accordingly, in the ovaries of the Hsd17b1-LacZ/Neo females, a marked decrease in the P4 concentration at estrus and during pseudopregnancy was observed. The reduced CL number was further confirmed by the reduced expression levels of several CL markers, especially *Hsd17b7* (Duan et al., 1997). E2 has been shown to stimulate luteal cell hypertrophy (McLean et al., 1990), and thus, it can be suggested that in the Hsd17b1-LacZ/Neo mice, a defect in granulosa cell luteinization causes the reduced P4 production. In the ovarian cycle, the expression of *Hsd17b1* is known to be upregulated during follicular growth; it is thereafter downregulated during luteinization and is not expressed in the CL. Thus, the coordinated expression of *Hsd17b1* during the early events of luteinization is essential for the maintenance and function of the CL.

6.2 Lack of HSD17B1 is compensated by another E2 producing enzyme, HSD17B7, in the ovarian steroid metabolism

As in the Hsd17b1-LacZ/Neo mice, puberty and estrous cycle were normal, and it is likely that some other enzyme or enzymes are responsible for producing the appropriate amount of E2 necessary for these processes to occur normally. A candidate for this role is HSD17B7 that has been shown to convert E1 to E2 and DHT to 3α-Adiol and 3β-Adiol. Furthermore, HSD17B7 has been shown to be essential in cholesterol biosynthesis, by catalyzing the conversion of zymosterone to zymos-

terol. In addition to being involved in these pathways, HSD17B7 has been identified as a prolactin receptor associated protein (Duan et al., 1997, 1996), and thus, it is potentially involved in the actions of prolactin signaling. HSD17B7 has also been shown to be a marker of large luteal cells originating from granulosa cells (Nokelainen et al., 2000; Parmer et al., 1992). However, studying the actions of this enzyme in ovarian physiology is very challenging, as the global knockout of the *Hsd17b7* gene results in embryonic lethality due to imbalanced sterol metabolism. The HSD17B7 enzyme has also been considered to be important in rodent pregnancy, as *Hsd17b7* is highly expressed in the ovaries of pregnant mice, specifically in the CL. Whether *HSD17B7* is also highly expressed in human pregnancy remains unknown. Evidently, cholesterol production is important in the ovary, as cholesterol is the precursor for steroids; the expression levels of *Hsd17b7* are markedly higher than the expression levels of other genes coding for cholesterol biosynthesis enzymes. This suggests that HSD17B7 has another metabolic role in the ovary in addition to its role in cholesterol biosynthesis. Whether the role of HSD17B7 is to produce E2 or to act in the prolactin receptor-mediated pathway remains unrevealed. The strong expression of *Hsd17b7* in the ovaries might also be responsible for the remaining (25 % in the WT mice) HSD17B activity measured in the proestrus ovaries. This is supported by the data showing that upregulation of the *Hsd17b7* gene is especially high during this cycle phase. Evidently, the compensation is not enough for the sophisticated steroid balance needed for the initiation or maintenance of pregnancy. In the ovaries of the pseudopregnant Hsd17b1-LacZ/Neo females, the expression of *Hsd17b7* was lost with a decrease in the number of CLs. As the two E2-producing enzymes HSD17B1 and HSD17B7 are absent in the ovaries of pseudopregnant Hsd17b1-LacZ/Neo mice, the ovaries might not be able to produce the amount of E2 needed for proper implantation or maintenance of pregnancy, which may explain the subfertility observed in the Hsd17b1-LacZ/Neo mice.

6.3 The fertility phenotype of the Hsd17b1-LacZ/Neo mice was caused by loss of HSD17B1 activity

One of the findings in the Hsd17b1-LacZ/Neo ovaries was the appearance of bundles of large granular cells in the ovarian stroma throughout the cycle and in pseudopregnancy. These structures were shown to be positive for the two lysosomal markers, LAMP1 and LIMP2. Lysosomal accumulation was also observed in the Naglu-Neo ovaries, and the accumulation was considered to be caused by the NAGLU deficiency in both the Hsd17b1-LacZ/Neo and Naglu-Neo knockout mouse lines. However, the Naglu-Neo female mice presented with a normal estrous cycle, fertility and litter size. In addition, the mRNA expression levels of

Hsd17b1, *Hsd17b7*, *Cyp17a1* and *Lhcgr*, which were altered in the Hsd17b1-LacZ/Neo mice, were not changed significantly in the ovaries of the Naglu-Neo pseudopregnant mice and the staining intensity for CYP17A1 was not different between the WT and Naglu-Neo mice. Based on these data, we conclude that subfertility in the Hsd17b1-LacZ/Neo mice is caused by a lack of HSD17B1 activity instead of a lack of NAGLU activity.

6.4 Deletion of *Hsd17b1* caused off-target effects

It is widely recognized, but rarely reported, that targeted disruption of the genome may disrupt neighboring genes and thus confuse the interpretation of the phenotypes observed. One of the concerns has been that the inserted antibiotic selection marker and its exogenous promoter in the targeting construct can either increase or decrease the expression levels of other genes located close to the insertion site (Olson et al., 1996; Pham et al., 1996). Furthermore, the targeting event may disrupt the regulatory and insulating elements within the targeted gene and thereby have effects on flanking genes (Lettice et al., 2002; Narboux-Nême et al., 2012; Zuniga et al., 2004). In this study, we revealed that deleting the whole 1.8-kb-long *Hsd17b1* gene from mice markedly affected transcription of the neighboring 5' gene, *Naglu*. Accordingly, severe downregulation of *Naglu* was observed in all tissues analyzed in the Hsd17b1-LacZ/Neo mice. The 3' end of the *Naglu* gene is located just 739 bp upstream from the transcriptional start site of the *Hsd17b1* gene. Therefore, we sequenced the critical parts of the *Hsd17b1* locus and confirmed that the sequence was intact between the *Naglu* exon 6 and the transcriptional start site of *Hsd17b1*. In addition, the 3' end of the targeting construct showed a proper targeting event, and the genomic region was clear of mis-targeting, deletions or mutations.

There are many intragenic regulatory elements found in the introns and in the 3' and 5' UTRs of genes that affect transcription. These elements can be secondary promoters, enhancers, repressors, and sequences coding for miRNAs (Barrett et al., 2012). Therefore, during gene targeting, there is a risk of unintended disruptions of expression of miRNAs encoded by regions located in the introns of the protein coding genes (Osokine et al., 2008). Moreover, the mouse *Hsd17b1* gene contains two short interspersed nuclear elements (SINEs) according to the WashU Epigenome Browser (http://epigenomegateway.wustl.edu/browser/). These short interspersed nuclear elements are believed to be integrated into a complex regulatory network capable of modifying gene expression across the eukaryotic genome, *e.g.*, by affecting the 3D structure of DNA or by directly interacting with transcrip-

tional repressors and activators (Goodrich and Kugel, 2006; Lee et al., 2016). Interestingly, the human *HSD17B1* gene also contains Alu sequences and cis-acting elements (Peltoketo et al., 1992a), but the genomic region coding for the HSD17B1 enzyme differs from that of the mouse. The sequence between the *HSD17B1* and *NAGLU* genes in humans extends up to 8.6 kb, as there is an untranscribed pseudogene between the two genes (Luu-The et al., 1990; Peltoketo et al., 1992a). There is a possibility that the observed linkage between the *Hsd17b1* and *Naglu* genes in mice is associated with the duplication of the *HSD17B1* gene in humans.

In addition, it is possible that the insertion of a targeting cassette can influence the regulation of neighboring genes in the mouse (Olson et al., 1996; Scacheri et al., 2001). Although the insertion cassette in the Hsd17b1-LacZ/Neo mice included a *neo* sequence under the *hUBC* promoter, removing the sequence from the targeted locus did not restore the expression of *Naglu*. The phenotype and *Naglu* expression were revealed to be similar in the mutant mice with or without the *neo* cassette. Furthermore, the insertion of the *neo* cassette into the Naglu-Neo mice did not disrupt transcription of the downstream *Hsd17b1* gene. By expressing the enzyme in the Hsd17b1-LacZ/Neo mice, we further confirmed that the reduced *Naglu* expression was not caused by the lack of HSD17B activity.

In the traditional view, genes are regulated by promoters and specific transcription factors, but it has become increasingly evident that regulation of eukaryotic genes is also dependent on their locations within the genome (Gierman et al., 2007). When the genome is modified by a large deletion, the modification may also interfere with co-regulation of neighboring genes, but this phenomenon does not explain the situation in the Hsd17b1-LacZ/Neo mice. Downregulation of *Naglu* was gene-specific, as the RNAseq data obtained from the ovary and adipose tissue showed that the expression of other genes located in the vicinity of the *Hsd17b1* was not similarly altered when the whole coding region of *Hsd17b1* gene was replaced with the targeting cassette.

6.5 The metabolic phenotype in Hsd17b1-LacZ/Neo mice was caused by the off-target *Naglu* deficiency

All mutant male mice with the whole *Hsd17b1* coding region deleted presented with reduced adiposity, increased lean mass, and liver steatosis. The metabolic phenotype in *Hsd17b1* knockout mice was found to be similar, but milder in females. Thus, the thesis on the metabolic phenotype was focusing on the males. The Hsd17b1-LacZ/Neo mice were tolerant to HFD-induced obesity and glucose tolerance was improved especially in the Hsd17b1-LacZ/Neo mice on HFD. These

observations could not be explained by food intake or increased locomotive activity. Thus, the mechanism was considered to be related to altered adipose tissue metabolism. To elucidate the effect of the lack of HSD17B1 activity on local steroid metabolism, the steroids in the gonadal white adipose tissue were analysed. Although, the *Hsd17b1* gene deletion caused alterations in the steroid levels in the female and male WAT, the changes were mild and not considered to underlie the strong phenotype of reduced adiposity. Similar to our results, decreased fat mass and decreased circulating glucose levels in the Hsd17b1-LaZ/Neo mice have been observed in investigations carried out by the International Mouse Phenotyping Consortium (IMPC, www.mousephenotype.org). Interestingly, in contrast to our data, the IMPC analyses demonstrated a decrease in the lean mass in Hsd17b1-LacZ/Neo mice. However, our RNA sequencing data clearly demonstrate that the neighboring gene, *Naglu*, is markedly downregulated in these mice. The lysosomal NAGLU enzyme is involved in the degradation of heparin sulfate, and reduced expression or a dysfunctional enzyme leads to the accumulation of heparan sulfate in the liver and kidney and to vacuolization in many cells, including macrophages, epithelial cells, and neurons (Li et al., 1999). Previous studies on mouse models with lysosomal dysfunction have shown that a profound adipose deficiency is a critical component of lysosomal dysfunction. Consequently, the *Naglu* knockout mice had significantly decreased fat mass and increased lean mass, as analyzed also by Woloszynek *et al*. (Woloszynek et al., 2007). Glucose tolerance was found to be improved in the Naglu-Neo mice, even though the liver was affected with increased weight associated with lipid and GAG accumulation. The metabolic phenotype of the Naglu-Neo mice was revealed to be highly similar to our observations with the Hsd17b1-LacZ/Neo and Hsd17b1-LacZ/Neo X HSD17B1TG mice, with the exception of the more severe lipid accumulation in the livers of the Hsd17b1-LacZ/Neo mice. Our observations of the very similar metabolic phenotypes strongly indicate that the phenotypes are mainly caused by the reduced expression of *Naglu* and are thus off-target effects in all *Hsd17b1* mutant mouse lines.

7 CONCLUSIONS

The hydroxysteroid (17beta) dehydrogenase (HSD17B) enzymes are important regulators of local androgen and estrogen metabolism in sex-steroid target tissues, *e.g.*, the ovary, testis and breast. In this study, we characterized the role of an estrogen biosynthesis enzyme, HSD17B1, in ovarian and adipose tissue physiology by using mouse models with the *Hsd17b1* gene deletion. However, a careful characterization of the *Hsd17b1* knockout mice revealed that the gene targeting method used in this study caused an off-target effect.

The main findings of the study are the following:

1. The HSD17B1 enzyme is needed for balanced steroidogenesis in ovaries. The data suggest that HSD17B1 regulates the estrogen ligand availability in theca cells, thus regulating *Cyp17a1* expression.

2. The HSD17B1 enzyme has an important role in luteinization and in initiation of pregnancy, as deletion of the *Hsd17b1* gene results in a reduced number of *corpora lutea* and in severe subfertility.

3. HSD17B1 is not a rate-limiting enzyme in ovarian estrogen synthesis in mice. The lack of the HSD17B1 enzyme does not alter the intraovarian estradiol level significantly. In addition, Hsd17b1-LacZ/Neo females reach puberty normally and have regular estrous cycles.

4. The genomic locus of *Hsd17b1* includes a strong regulatory element that is essential for *Naglu* expression. Replacement of the whole coding region of *Hsd17b1* with the LacZ/Neo cassette results in globally reduced expression of a neighboring gene, *Naglu*. The defect in the NAGLU enzyme, which causes lysosomal accumulation in the stroma of the ovary, does not affect female fertility.

5. The off-target effect observed is mainly responsible for the metabolic phenotype present in the Hsd17b1-LacZ/Neo mice described in the present study and on the International Knockout Mouse Consortium website (www.mousephenotype.org).

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REFERENCES

Abuin, A., Hansen, G.M., and Zambrowicz, B. (2007). Gene Trap Mutagenesis. In Handbook of Experimental Pharmacology, R. Feil, and D. Metzger, eds. (Springer), pp. 129–147.

Adamski, J., Husen, B., Marks, F., and Jungblut, P.W. (1992). Purification and properties of oestradiol 17 beta-dehydrogenase extracted from cytoplasmic vesicles of porcine endometrial cells. Biochem. J. *288*, 375–381.

Adamski, J., Cartensen, J., Husen, B., Kaufmann, M., DeLaunoit, Y., Leenders, F., Markus, M., and Jungblut, P.W. (1996). New 17beta-Hydrogenases, Molecular and Cell Biology of the Type IV Porcine and Human Enzymes. Ann. New York Acad. Sci. *784*, 124–136.

Akinola, L., Poutanen, M., Vihko, R., and Vihko, P. (1997). Pregnant Rats. Endocrinology *138*, 2886–2892.

Allen, N., Cran, D., Barton, S., Hettle, S., Reik, W., and Surani, M.A. (1988). Transgenes as probes for active chromosomal domains in mouse development. Nature *333*, 852–855.

Andrade, F., Aldamiz-Echevarria, L., Llarena, M., and Couce, M.L. (2015). Sanfilippo syndrome: Overall review. Pediatr. Int. *57*, 331–338.

Aziz, N., Maxwell, M.M., St-Jacques, B., and Brenner, B.M. (1993). Downregulation of Ke 6, a novel gene encoded within the major histocompatibility complex, in murine polycystic kidney disease. Mol Cell Biol. *13*, 1847–1853.

Baerga, R., Zhang, Y., Chen, P.H., Goldman, S., and Jin, S. (2009). Targeted deletion of autophagy-related 5 (atg5) impairs adipogenesis in a cellular model and in mice. Autophagy *5*, 1118–1130.

Baes, M., Huyghe, S., Carmeliet, P., Declercq, P.E., Collen, D., Mannaerts, G.P., and Van Veldhoven, P.P. (2000). Inactivation of the peroxisomal multifunctional protein-2 in mice impedes the degradation of not only 2-methyl-branched fatty acids and bile acid intermediates but also of very long chain fatty acids. J. Biol. Chem. *275*, 16329–16336.

Bak, B., Carpio, L., Kipp, J.L., Lamba, P., Wang, Y., Ge, R.S., Hardy, M.P., Mayo, K.E., and Bernard, D.J. (2009). Activins regulate 17-beta-hydroxysteroid dehydrogenase type I transcription in murine gonadotrope cells. J. Endocrinol. *201*, 89–104.

Barrett, L.W., Fletcher, S., and Wilton, S.D. (2012). Regulation of eukaryotic gene expression by the untranslated gene regions and other non-coding elements. Cell. Mol. Life Sci. *69*, 3613–3634.

Bauman, D.R., Steckelbroeck, S., Williams, M. V., Peehl, D.M., and Penning, T.M. (2006). Identification of the Major Oxidative 3α-Hydroxysteroid Dehydrogenase in Human Prostate That Converts 5α-Androstane-3α,17β-diol to 5α-Dihydrotestosterone: A Potential Therapeutic Target for Androgen-Dependent Disease. Mol. Endocrinol. *20*, 444–458.

Beck, K., Kaserer, T., Schuster, D., and Odermatt, A. (2017). Virtual screening applications in short-chain dehydrogenase/reductase research. J. Steroid Biochem. Mol. Biol. *171*, 157–177.

Beesley, C.E., Jackson, M., Young, E.P., Vellodi, A., and Winchester, B.G. (2005). Molecular defects in Sanfilippo syndrome type B (mucopolysaccharidosis IIIB). J. Inherit. Metab. Dis. *28*, 759–767.

Behringer, R., Gertsenstein, M., Vintersen Nagy, K., and Nagy, A. (2014). Manipulating the Mouse Embryo: A Laboratory Manual, Fourth Edition. In Cold Harbor Laboratory Press, pp. 237–460.

Beilstein, F., Bouchoux, J., Rousset, M., and Demignot, S. (2013). Proteomic Analysis of Lipid Droplets from Caco-2/TC7 Enterocytes Identifies Novel Modulators of Lipid Secretion. PLoS One *8*, 1–17.

Bellemare, V., Laberge, P., Noël, S., Tchernof, A., and Luu-The, V. (2009). Differential estrogenic 17beta-hydroxysteroid dehydrogenase activity and type 12 17beta-hydroxysteroid dehydrogenase expression levels in preadipocytes and differentiated adipocytes. J. Steroid Biochem. Mol. Biol. *114*, 129–134.

Bellin, M.E., Ax, R.L., Laufer, N., Tarlatzis, B.C., DeCherney, A.H., Feldberg, D., and Haseltine, F.P. (1986). Glycosaminoglycans in follicular fluid from women undergoing in vitro fertilization and their relationship to cumulus expansion, fertilization, and development. Fertil. Steril. *45*, 244–248.

Bhaumik, M., Muller, V.J., Rozaklis, T., Johnson, L., Dobrenis, K., Bhattacharyya, R., Wurzelmann, S., Finamore, P., Hopwood, J.J., Walkley, S.U., et al. (1999). A mouse model for mucopolysaccharidosis type III A (Sanfilippo syndrome). Glycobiology *9*, 1389–1396.

Biswas, M.G., and Russell, D.W. (1997). Expression cloning and characterization of oxidative 17beta- and 3alpha-hydroxysteroid dehydrogenases from rat and human prostate. J. Biol. Chem. *272*, 15959–15966.

Blanchard, P.G., and Luu-The, V. (2007). Differential androgen and estrogen substrates specificity in the mouse and primates type 12 17beta-hydroxysteroid dehydrogenase. J. Endocrinol. *194*, 449–455.

Bleau, G., Roberts, K.D., and Chapdelaine, A. (1974). The in vitro and in vivo uptake and metabolism of steroids in human adipose tissue. J. Clin. Endocrinol. Metab. *39*, 236–246.

Bouabe, H., and Okkenhaug, K. (2013). Gene Targeting in Mice: a Review. Methods Mol Biol *1064*, 315–336.

Breitling, R., Krazeisen, A., Moller, G., and Adamski, J. (2001). 17Beta-Hydroxysteroid Dehydrogenase Type 7-an Ancient 3-Ketosteroid Reductase of Cholesterogenesis. Mol. Cell. Endocrinol. *171*, 199–204.

Byers, S.L., Wiles, M. V., Dunn, S.L., and Taft, R.A. (2012). Mouse estrous cycle identification tool and images. PLoS One *7*, 1–5.

Caligioni, C.S. (2009). Assessing reproductive status/stages in mice. Curr. Protoc. Neurosci. *4*, 1–8.

Campbell, K.L., Foster-Schubert, K.E., Makar, K.W., Kratz, M., Hagman, D., Schur, E.A., Habermann, N., Horton, M., Abbenhardt, C., Kuan, L.Y., et al. (2013). Gene expression changes in adipose tissue with diet- and/or exercise-induced weight loss. Cancer Prev Res *6*, 217–231.

Casey, M.L., MacDonald, P.C., and Andersson, S. (1994). 17beta-Hydroxysteroid Dehydrogenase Type 2: Chromosomal Assignment and Progestin Regulation of Gene Expression in Human Endometrium. J Clin Invest *50*, 3–11.

Cheah, S., and Behringer, R. (2000). Gene-targeting strategies. Methods Mol. Biol. *136*, 455–463.

Chen, C., Krohn, J., Bhattacharya, S., and Davies, B. (2011). A comparison of exogenous promoter activity at the ROSA26 locus using a PhiC31 integrase mediated cassette exchange approach in mouse es cells. PLoS One *6*, 6–13.

Chen, T., McClellan, M., Diekman, M., Abel, J., and Niswender, G. (1977). Localization of Human Chorionic Gonadotropin in Lysosomes of Ovine Luteal Cells. In Structure and Function of the Gonadotrophins, (Springer), pp. 591–612.

Chen, Z., Kastaniotis, A.J., Miinalainen, I.J., Rajaram, V., Wierenga, R.K., and Hiltunen, J.K. (2009). 17beta-hydroxysteroid dehydrogenase type 8 and carbonyl reductase type 4 assemble as a ketoacyl reductase of human mitochondrial FAS. FASEB J. *23*, 3682–3691.

Clarke, H.G., Hope, S.A., Byers, S., and Rodgers, R.J. (2006). Formation of ovarian follicular fluid may be due to the osmotic potential of large glycosaminoglycans and proteoglycans. Reproduction *132*, 119–131.

Colette, S., Defrère, S., Van Kerk, O., Van Langendonckt, A., Dolmans, M.M., and Donnez, J. (2013). Differential expression of steroidogenic enzymes according to endometriosis type. Fertil. Steril. *100*, 1642–1649.

Corbould, A., Bawden, M., Lavranos, T., Rodgers, R., and Judd, S. (2002). The effect of obesity on the ratio of type 3 17beta-hydroxysteroid dehydrogenase mRNA to cytochrome P450 aromatase mRNA in subcutaneous abdominal and intra-abdominal adipose tissue of women. Int J Obes Relat Metab Disord *26*, 165–175.

Couse, J.F., Yates, M.M., Walker, V.R., and Korach, K.S. (2003). Characterization of the hypothalamic-pituitary-gonadal axis in estrogen receptor (ER) null mice reveals hypergonadism and endocrine sex reversal in females lacking ERalpha but not ERbeta. Mol. Endocrinol. *17*, 1039–1053.

Cuervo, a M., Knecht, E., Terlecky, S.R., and Dice, J.F. (1995). Activation of a selective pathway of lysosomal proteolysis in rat liver by prolonged starvation. Am. J. Physiol. *269*, C1200–C1208.

Dalla Valle, L., Toffolo, V., Nardi, A., Fiore, C., Bernante, P., Di Liddo, R., Parnigotto, P.P., and Colombo, L. (2006). Tissue-specific transcriptional initiation and activity of steroid sulfatase complementing dehydroepiandrosterone sulfate uptake and intracrine steroid activations in human adipose tissue. J. Endocrinol. *190*, 129–139.

Van Deerlin, P.G., Cekleniak, N., Coutifaris, C., Boyd, J., and Strauss, J.F. (1997). Evidence for the oligoclonal origin of the granulosa cell population of the mature human follicle. J. Clin. Endocrinol. Metab. *82*, 3019–3024.

Dieuaide-Noubhani, M., Novikov, D., Baumgart, E., Vanhooren, J.C., Fransen, M., Goethals, M., Vandekerckhove, J., Van Veldhoven, P.P., and Mannaerts, G.P. (1996). Further characterization of the peroxisomal 3-hydroxyacyl-CoA dehydrogenases from rat liver. Relationship between the different dehydrogenases and evidence that fatty acids and the C27 bile acids di- and tri-hydroxycoprostanic acids are metabolized by sep. Eur. J. Biochem. *240*, 660–666.

Dieuaide-Noubhani, M., Asselberghs, S., Mannaerts, G.P., and Van Veldhoven, P.P. (1997). Evidence that multifunctional protein 2, and not multifunctional protein 1, is involved in

the peroxisomal beta-oxidation of pristanic acid. Biochem. J. *325 (Pt 2)*, 367–373.

Dong, H., and Czaja, M.J. (2011). Regulation of lipid droplets by autophagy. Trends Endocrinol. Metab. *22*, 234–240.

Duan, W., Parmer, T., Albarracin, C., Zhong, L., and Gibori, G. (1997). PRAP, a Prolactin Receptor Associated Protein: Its Gene Expression and Regulation in the Corpus Luteum 1. Endocrinology *138*, 3216–3221.

Duan, W.R., Linzer, D.I.H., and Gibori, G. (1996). Cloning and characterization of an ovarian-specific protein that associates with the short form of the prolactin receptor. J. Biol. Chem. *271*, 15602–15607.

Duerrschmidt, N., Zabirnyk, O., Nowicki, M., Ricken, A., Hmeidan, F.A., Blumenauer, V., Borlak, J., and Spanel-Borowski, K. (2006). Lectin-like oxidized low-density lipoprotein receptor-1-mediated autophagy in human granulosa cells as an alternative of programmed cell death. Endocrinology *147*, 3851–3860.

Ezaki, J., Matsumoto, N., Takeda-Ezaki, M., Komatsu, M., Takahashi, K., Hiraoka, Y., Taka, H., Fujimura, T., Takehana, K., Yoshida, M., et al. (2011). Liver autophagy contributes to the maintenance of blood glucose and amino acid levels. Autophagy *7*, 727–736.

Fabbrini, E., Sullivan, S., and Klein, S. (2010). Obesity and Nonalcoholic Fatty Liver Disease: Biochemical, Metabolic and Clinical Implications. Hepatology *51*, 679–689.

von Figura, K. (1977). Human a-N-Acetylglucosaminidase. Eur J Biochem *80*, 535–542.

Fomitcheva, J., Baker, M.E., Anderson, E., Lee, G.Y., and Aziz, N. (1998). Characterization of Ke 6, a new 17beta-hydroxysteroid dehydrogenase, and its expression in gonadal tissues. J. Biol. Chem. *273*, 22664–22671.

Foryst-Ludwig, A., and Kintscher, U. (2010). Metabolic impact of estrogen signalling through ERalpha and ERbeta. J. Steroid Biochem. Mol. Biol. *122*, 74–81.

Fouad Mansour, M., Pelletier, M., Boulet, M.M., Mayrand, D., Brochu, G., Lebel, S., Poirier, D., Fradette, J., Cianflone, K., Luu-The, V., et al. (2015). Oxidative activity of 17β-hydroxysteroid dehydrogenase on testosterone in male abdominal adipose tissues and cellular localization of 17β-HSD type 2. Mol Cell Endocrinol *414*, 168–176.

Fournet-Dulguerov, N., MacLusky, N., Leranth, C., Todd, R., Mendelson, C.R., Simpson, E.R., and Naftolin, F. (1987). Immunohistochemical Localization of Aromatase Cytochrome P-450 and Estradiol Dehydrogenase in the Syncytiotrophoblast of the Human Placenta. J. Clin. Endocrinol. Metab. *65*, 757–764.

Freeman, M.E., and Neill, J.D. (1972). The pattern of prolactin secretion during pseudopregnancy in the rat: A daily nocturnal surge. Endocrinology *90*, 1292–1294.

Freeman, M.E., Smith, M.S., Nazian, S.J., and Neill, J.D. (1974). Ovarian and Hypothalamic Control of the Daily Surges of Prolactin Secretion During Pseudopregnancy in the Rat. Endocrinology *94*, 875–882.

Friedrich, G., and Soriano, P. (1991). Promoter traps in embryonic stem cells: a genetic screen to identify and mutant developmental genes in mice. Genes Dev. *5*, 1513–1523.

Gawriluk, T.R., Hale, A.N., Flaws, J.A., Dillon, C.P., Green, D.R., and Rucker, E.B. (2011). Autophagy is a cell survival program for female germ cells in the murine ovary. Reproduction *141*, 759–765.

Gaytán, M., Morales, C., Sánchez-Criado, J.E., and Gaytán, F. (2008). Immunolocalization of beclin 1, a bcl-2-binding, autophagy-related protein, in the human ovary: Possible relation to life span of corpus luteum. Cell Tissue Res. *331*, 509–517.

Geloen, A., Roy, P.E., and Bukowiecki, J. (1989). Regression of white adipose tissue in diabetic rats. Am J Physiol *257*, 547–553.

Ghersevich, S., Poutanen, M., Tapanainen, J., and Vihko, R. (1994a). Hormonal Regulation of Rat 17beta Hydroxysteroid Dehydrogenase Type 1 in Cultured Rat Granulosa Cells: Effects of Recombinant Follicle-Stimulating Hormone, Estrogens, androgens, and Epidermal Growth Factor. Endocrinology *135*, 1963–1971.

Ghersevich, S., Poutanen, M., Martikainen, H., and Vihko, R. (1994b). Expression of 17 beta-hydroxysteroid dehydrogenase in human granulosa cells: correlation with follicular size, cytochrome P450 aromatase activity and oestradiol production. J. Endocrinol. *143*, 139–150.

Ghersevich, S., Nokelainen, P., Poutanen, M., Orava, M., Autio-Harmainen, H., Rajaniemi, H., and Vihko, R. (1994c). Rat 17beta-Hydroxysteroid Dehydrogenase Type 1: Primary Structure and Regulation of Enzyme Expression in Rat Ovary by Diethylstilbestrol Gonadotropins in Vivo. Endocrinology *135*, 1477–1487.

Gierman, H.J., Indemans, M.H.G., Koster, J., Goetze, S., Seppen, J., Geerts, D., Driel, R. Van, and Versteeg, R. (2007). Domain-wide regulation of gene expression in the human genome. Genome Res. *17*, 1286–1295.

Gittens, J.E., Mhawi, A.A., Lidington, D., Ouellette, Y., and Kidder, G.M. (2003). Functional analysis of gap junctions in ovarian granulosa cells: distinct role for connexin43 in early stages of folliculogenesis. Am J Physiol Cell Physiol *284*, C880-887.

Gluchowski, N.L., Becuwe, M., Walther, T.C., and Farese, R. V. (2017). Lipid droplets and liver disease: from basic biology to clinical implications. Nat. Rev. Gastroenterol. Hepatol. *14*, 343–355.

Goldman, S.J., Zhang, Y., and Jin, S. (2011). Autophagic degradation of mitochondria in white adipose tissue differentiation. Antioxid. Redox Signal. *14*, 1971–1978.

Goodrich, J.A., and Kugel, J.F. (2006). Non-coding-RNA regulators of RNA polymerase II transcription. Nat. Rev. Mol. Cell Biol. *7*, 612–616.

Gossler, A., Joyner, A.L., Rossant, J., and Skarnes, W.C. (1988). Mouse Embryonc Stem Cells and Reporter Constructs. Science *244*, 463–465.

Guan, C., Ye, C., Yang, X., and Gao, J. (2010). A review of current large-scale mouse knockout efforts. Genesis *48*, 73–85.

Haas, D., and Muenke, M. (2010). Abnormal sterol metabolism in holoprosencephaly. Am. J. Med. Genet. Part C Semin. Med. Genet. *154*, 102–108.

Hadjantonakis, A.-K., Pirity, M., and Nagy, A. (2008). Cre Recombinase Mediated Alterations of the Mouse Genome Using Embryonic Stem Cells. Nature *216*, 204–204.

Haynes, B.P., Straume, A.H., Geisler, J., A'Hern, R., Helle, H., Smith, I.E., Lønning, P.E., and Dowsett, M. (2010). Intratumoral estrogen disposition in breast cancer. Clin. Cancer Res. *16*, 1790–1801.

He, X.-Y., and Yang, S.-Y. (2006). Roles of type 10 17beta-hydroxysteroid dehydrogenase in intracrinology and metabolism of isoleucine and fatty acids. Endocr. Metab. Immune Disord. Drug Targets *6*, 95–102.

He, X., Merz, G., Mehta, P., Schulz, H., and Yang, S. (1999). Human Brain Short Chain L -3-Hydroxyacyl Coenzyme A Dehydrogenase Is a Single-domain Multifunctional Enzyme. Biochemistry *274*, 15014–15019.

He, X.Y., Schulz, H., and Yang, S.Y. (1998). A human brain L-3-hydroxyacyl coenzyme a dehydrogenase is identical to an amyloid p-peptide-binding protein involved in disease,alzheimers. J. Biol. Chem. *273*, 10741–10746.

Hemberger, M., and Dean, W. (2014). First Cell Fate Decisions in Early Development. In The Guide to Investigation of Mouse Pregnancy, (Elsevier), pp. 95–106.

Holwerda, S., and de Laat, W. (2012). Chromatin loops, gene positioning, and gene expression. Front. Genet. *3*, 1–13.

Horiguchi, Y., Araki, M., and Motojima, K. (2008). 17beta-Hydroxysteroid dehydrogenase type 13 is a liver-specific lipid droplet-associated protein. Biochem. Biophys. Res. Commun. *370*, 235–238.

Huang, X.F., and Luu-The, V. (2000). Molecular characterization of a first human 3(alpha-->beta)-hydroxysteroid epimerase. J. Biol. Chem. *275*, 29452–29457.

Huhtinen, K., Desai, R., Ståhle, M., Salminen, A., Handelsman, D.J., Perheentupa, A., and Poutanen, M. (2012). Endometrial and endometriotic concentrations of estrone and estradiol are determined by local metabolism rather than circulating levels. J. Clin. Endocrinol. Metab. *97*, 4228–4235.

Huhtinen, K., Saloniemi-Heinonen, T., Keski-Rahkonen, P., Desai, R., Laajala, D., Ståhle, M., Häkkinen, M.R., Awosanya, M., Suvitie, P., Kujari, H., et al. (2014). Intra-tissue steroid profiling indicates differential progesterone and testosterone metabolism in the endometrium and endometriosis lesions. J. Clin. Endocrinol. Metab. *99*, E2188–E2197.

Huyghe, S., Mannaerts, G.P., Baes, M., and Van Veldhoven, P.P. (2006). Peroxisomal multifunctional protein-2: The enzyme, the patients and the knockout mouse model. Biochim. Biophys. Acta - Mol. Cell Biol. Lipids *1761*, 973–994.

Jansson, A. (2009). 17Beta-hydroxysteroid dehydrogenase enzymes and breast cancer. J. Steroid Biochem. Mol. Biol. *114*, 64–67.

Jansson, A.K., Gunnarsson, C., Cohen, M., Sivik, T., and Stål, O. (2006). 17beta-Hydroxysteroid Dehydrogenase 14 Affects Estradiol Levels in Breast Cancer Cells and Is a Prognostic Marker in Estrogen Receptor-Positive Breast Cancer. Cancer Res. *66*, 11471–11477.

Jarabak, J., Adams, J.A., Williams-Ashman, H.G., and Talalay, P. (1962). Purification of a 17β-Hydroxysteroid Dehydrogenase of Human Placenta and Studies on Its Transhydrogenase Function. J. Biol. Chem. *237*, 345–357.

Jin, Y., Eser, U., Struhl, K., and Churchman, L.S. (2017). The Ground State and Evolution of Promoter Region Directionality. Cell *170*, 889–898.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., and Charpentier, E. (2012). A Programmable Dual-RNA – Guided DNA

Endonuclease in Adaptive Bacterial Immunity. Science *337*, 816–822.

Jokela, H., Rantakari, P., Lamminen, T., Strauss, L., Ola, R., Mutka, A.L., Gylling, H., Miettinen, T., Pakarinen, P., Sainio, K., et al. (2010). Hydroxysteroid (17beta) dehydrogenase 7 activity is essential for fetal de novo cholesterol synthesis and for neuroectodermal survival and cardiovascular differentiation in early mouse embryos. Endocrinology *151*, 1884–1892.

Kaminski, T., Akinola, L., Poutanen, M., Vihko, R., and Vihko, P. (1997). Growth factors and phorbol-12-myristate-13-acetate modulate the follicle-stimulating hormone- and cyclic adenosine-3',5'-monophosphate-dependent regulation of 17beta-hydroxysteroid dehydrogenase type 1 expression in rat granulosa cells. Mol Cell Endocrinol *136*, 47–56.

Kaushik, S., and Cuervo, A.M. (2012). Chaperone-mediated autophagy: A unique way to enter the lysosome world. Trends Cell Biol. *22*, 407–417.

Kelly, D.M., Nettleship, J.E., Akhtar, S., Muraleedharan, V., Sellers, D.J., Brooke, J.C., McLaren, D.S., Channer, K.S., and Jones, T.H. (2014). Testosterone suppresses the expression of regulatory enzymes of fatty acid synthesis and protects against hepatic steatosis in cholesterol-fed androgen deficient mice. Life Sci. *109*, 95–103.

Kemiläinen, H., Adam, M., Mäki-Jouppila, J., Damdimopoulou, P., Damdimopoulos, A., Kere, J., Hovatta, O., Laajala, T., Aittokallio, T., Adamski, J., et al. (2016). The hydroxysteroid (17beta) dehydrogenase family gene HSD17B12 is involved in the prostaglandin synthesis pathway, the ovarian function, and regulation of fertility. Endocrinology *157*, 3719–3730.

Kleinjan, D.A., and van Heyningen, V. (2005). Long-range control of gene expression: emerging mechanisms and disruption in disease. Am. J. Hum. Genet. *76*, 8–32.

Klionsky, D.J. (2005). The molecular machinery of autophagy: unanswered questions. J. Cell Sci. *118*, 7–18.

Knuuttila, M., Yatkin, E., Kallio, J., Savolainen, S., Laajala, T.D., Aittokallio, T., Oksala, R., Häkkinen, M., Keski-Rahkonen, P., Auriola, S., et al. (2014). Castration induces up-regulation of intratumoral androgen biosynthesis and androgen receptor expression in an orthotopic VCaP human prostate cancer xenograft model. Am. J. Pathol. *184*, 2163–2173.

Koyanagi, K.O., Hagiwara, M., Itoh, T., Gojobori, T., and Imanishi, T. (2005). Comparative genomics of bidirectional gene pairs and its implications for the evolution of a transcriptional regulation system. Gene *353*, 169–176.

Kramerov, D.A., and Vassetzky, N.S. (2011). SINEs. Wiley Interdiscip. Rev. RNA *2*, 772–786.

Kuk, J.L., Lee, S., Heymsfield, S.B., and Ross, R. (2005). Waist circumference and abdominal adipose tissue distribution: influence of age and sex. Am. J. Clin. Nutr. *81*, 1330–1334.

Kunzt, J.B., Schwarz, H., and Mayer, A. (2004). Determination of Four Sequential Stages during Microautophagy in Vitro. J. Biol. Chem. *279*, 9987–9996.

Labrie, F. (2015). All sex steroids are made intracellularly in peripheral tissues by the mechanisms of intracrinology after menopause. J. Steroid Biochem. Mol. Biol. *145*, 133–138.

Labrie, C., and Belanger, A. (1988). Androgenic Activity oí Debydroepiandrosterone Androstenedione in tbe Rat Ventral Prostate. J. Steroid Biochem. Mol. Biol. *123*, 1412–1417.

Labrie, Y., Durocher, F., Lachance, Y., Turgeon, C., Simard, J., Labrie, C., and Labrie, F. (1995). The human type II 17 beta-hydroxysteroid dehydrogenase gene encodes two alternatively spliced mRNA species. DNA Cell Biol. *14*, 849–861.

Langer, L.J., and Engel, L.L. (1958). Human placental estradiol-17 beta dehydrogenase. I. Concentration, characterization and assay. J. Biol. Chem. *233*, 583–588.

Laplante, Y., Rancourt, C., and Poirier, D. (2009). Relative involvement of three 17beta-hydroxysteroid dehydrogenases (types 1, 7 and 12) in the formation of estradiol in various breast cancer cell lines using selective inhibitors. Mol. Cell. Endocrinol. *301*, 146–153.

Laubner, D., Breitling, R., and Adamski, J. (2003). Embryonic expression of cholesterogenic genes is restricted to distinct domains and colocalizes with apoptotic regions in mice. Mol. Brain Res. *115*, 87–92.

Lee, H., Ayarpadikannan, S., and Kim, H. (2016). Role of transposable elements in genomic rearrangement, evolution, gene regulation and epigenetics in primates. Genes Genet. Syst. *90*, 245–257.

Lettice, L. a, Horikoshi, T., Heaney, S.J.H., van Baren, M.J., van der Linde, H.C., Breedveld, G.J., Joosse, M., Akarsu, N., Oostra, B. a, Endo, N., et al. (2002). Disruption of a long-range cis-acting regulator for Shh causes preaxial polydactyly. Proc Natl Acad Sci USA *99*, 7548–7553.

Levine, J.E. (2015). Neuroendocrine Control of the Ovarian Cycle of the Rat. In Knobil and Neill's Physiology of Reproduction, (Elsevier), pp. 1199–1257.

Li, H.H., Yu, W.H., Rozengurt, N., Zhao, H.Z., Lyons, K.M., Anagnostaras, S., Fanselow, M.S., Suzuki, K., Vanier, M.T., and Neufeld, E.F. (1999). Mouse model of Sanfilippo syndrome type B produced by targeted disruption of the gene encoding alpha-N-acetylglucosaminidase. Proc Natl Acad Sci USA *96*, 14505–14510.

Li, J., Papadopoulos, V., and Vihma, V. (2015). Steroid biosynthesis in adipose tissue. Steroids *103*, 89–104.

Lin, H.-Y., Yu, I.-C., Wang, R.-S., Chen, Y.-T., Liu, N.-C., Altuwaijri, S., Hsu, C.-L., Ma, W.-L., Jokinen, J., Sparks, J.D., et al. (2008). Increased hepatic steatosis and insulin resistance in mice lacking hepatic androgen receptor. Hepatology *47*, 1924–1935.

Lin, J.M., Collins, P.J., Trinklein, N.D., Fu, Y., Xi, H., Myers, R.M., Weng, Z., Lin, J.M., Collins, P.J., Trinklein, N.D., et al. (2007). Transcription factor binding and modified histones in human bidirectional promoters Transcription factor binding and modified histones in human bidirectional promoters. Genome Res. *17*, 818–827.

Lin, S.-X., Poirier, D., and Adamski, J. (2013). A challenge for medicinal chemistry by the 17β-hydroxysteroid dehydrogenase superfamily: an integrated biological function and inhibition study. Curr. Top. Med. Chem. *13*, 1164–1171.

Liu, S., Huang, C., Li, D., Ren, W., Zhang, H., Qi, M., Li, X., and Yu, L. (2007). Molecular cloning and expression analysis of a new gene for short-chain dehydrogenase/reductase 9. Acta Biochim. Pol. *54*, 213–218.

Livingstone, D.E.W., Barat, P., Di Rollo, E.M., Rees, G.A., Weldin, B.A., Rog-Zielinska, E.A., Macfarlane, D.P., Walker, B.R., and Andrew, R. (2015). 5alpha-Reductase type 1 deficiency or inhibition predisposes to insulin resistance, hepatic steatosis, and liver fibrosis in rodents. Diabetes *64*, 447–458.

Loriot, Y., Fizazi, K., Jones, R.J., Van Den Brande, J., Molife, R.L., Omlin, A., James, N.D., Baskin-Bey, E., Heeringa, M., Baron, B., et al. (2014). Safety, tolerability and anti-tumour activity of the androgen biosynthesis inhibitor ASP9521 in patients with metastatic castration-resistant prostate cancer: Multi-centre phase I/II study. Invest. New Drugs *32*, 995–1004.

Lukacik, P., Kavanagh, K.L., and Oppermann, U. (2006). Structure and function of human 17beta-hydroxysteroid dehydrogenases. Mol. Cell. Endocrinol. *248*, 61–71.

Luu-The, V., Labrie, C., Zhao, H.F., Couet, J., Lachance, Y., Simard, J., Leblanc, G., Cote, J., Berube, D., Gagne, R., et al. (1989). Characterization of cDNAs for Human Estradiol 17/beta-Dehydrogenase and Assignment of the Gene to Chromosome 17: Evidence of two mRNA Species with Distinct 5'-Termini in Human Placenta. Mol. Endocrinol. *3*, 1301–1309.

Luu-The, V., Labrie, C., Simard, J., Lachance, Y., Zhao, H.F., Couet, J., Leblanc, G., and Labrie, F. (1990). Structure of two in tandem human 17 beta-hydroxysteroid dehydrogenase genes. Mol. Endocrinol. *4*, 268–275.

Luu-The, V., Tremblay, P., and Labrie, F. (2006). Characterization of type 12 17beta-hydroxysteroid dehydrogenase, an isoform of type 3 17beta-hydroxysteroid dehydrogenase responsible for estradiol formation in women. Mol. Endocrinol. *20*, 437–443.

Ma, W., Song, H., Das, S.K., Paria, B.C., and Dey, S.K. (2003). Estrogen is a critical determinant that specifies the duration of the window of uterine receptivity for implantation. Proc Natl Acad Sci USA *100*, 2963–2968.

Malarkey, D.E., Johnson, K., Ryan, L., Boorman, G., and Maronpot, R.R. (2005). New Insights into Functional Aspects of Liver Morphology. Toxicol. Pathol. *33*, 27–34.

Mansour, S.L., Thomas, K.R., and Capecchi, M.R. (1988). Disruption of the proto-oncogene int-2 in mouse embryo-derived stem cells: a general strategy for targeting mutations to non-selectable genes. Nature *336*, 348–352.

Marchais-Oberwinkler, S., Henn, C., Möller, G., Klein, T., Negri, M., Oster, A., Spadaro, A., Werth, R., Wetzel, M., Xu, K., et al. (2011). 17beta-Hydroxysteroid dehydrogenases (17beta-HSDs) as therapeutic targets: Protein structures, functions, and recent progress in inhibitor development. J. Steroid Biochem. Mol. Biol. *125*, 66–82.

Marcó, S., Pujol, A., Roca, C., Motas, S., Ribera, A., Garcia, M., Molas, M., Villacampa, P., Melia, C.S., Sánchez, V., et al. (2016). Progressive neurologic and somatic disease in a novel model of human Mucopolysaccharidosis type IIIC. Dis. Model. Mech. *9*, 999–1013.

Marijanovic, Z., Laubner, D., Moller, G., Gege, C., Husen, B., Adamski, J., and Breitling, R. (2003). Closing the gap: identification of human 3-ketosteroid reductase, the last unknown enzyme of mammalian cholesterol biosynthesis. Mol. Endocrinol. *17*, 1715–1725.

Markus, M., Husen, B., and Adamski, J. (1995). The Subcellular Localization of 17beta-Hydroxysteroid Type 4 and its Interaction with Actin. J Steroid Biochem Mol Biol *55*, 617–621.

Mason, H., Willis, D., Beard, R., Winston, R., Margara, R., and Franks, S. (1994). Estradiol Production by Granulosa Cells of Normal and Polycystic Ovaries: Relationship to Menstrual Cycle History and Concentrations of Gonadotropins and Sex Steroids in Follicular Fluid. J. Clin. Endocrinol. Metab. *79*, 1355–1360.

Mauvais-Jarvis, F. (2011). Estrogen and androgen receptors: Regulators of fuel homeostasis and emerging targets for diabetes and obesity. Trends Endocrinol. Metab. *22*, 24–33.

Mauvais-Jarvis, F., Clegg, D.J., and Hevener, A.L. (2013). The role of estrogens in control of energy balance and glucose homeostasis. Endocr Rev *34*, 309–338.

Maxwell, M.M., Nearing, J., and Aziz, N. (1995). Ke 6 gene. Sequence and organization and aberrant regulation in murine polycystic kidney disease. J Biol Chem *270*, 25213–25219.

McLean, M., Khan, I., Puryear, T., and G, G. (1990). Induction and repression of specific estradiol sensitive proteins in the rat corpus luteum. Chin J Physiol *33*, 353–366.

McNatty, K., Makris, A., DeGrazia, C., Osathanondh, R., and Ryan, K.J. (1979). The Production of Progesterone, Androgens, and Estrogens by Granulosa Cells, Thecal Tissue, and Stromal Tissue from Human Ovaries in Vitro. J. Clin. Endocrinol. Metab. *49*, 687–699.

McTernan, P.G., Anderson, L.A., Anwar, A.J., Eggo, M.C., Crocker, J., Barnett, A.H., Stewart, P.M., and Kumar, S. (2002). Glucocorticoid regulation of P450 aromatase activity in human adipose tissue: gender and site differences. J. Clin. Endocrinol. Metab. *87*, 1327–1336.

Meier, I.D., Bernreuther, C., Tilling, T., Neidhardt, J., Wong, Y.W., Schulze, C., Streichert, T., and Schachner, M. (2010). Short DNA sequences inserted for gene targeting can accidentally interfere with off-target gene expression. FASEB J. *24*, 1714–1724.

Miettinen, M., Isomaa, V., Peltoketo, H., Ghosh, D., and Vihko, P. (2000). Estrogen metabolism as a regulator of estrogen action in the mammary gland. J. Mammary Gland Biol. Neoplasia *5*, 259–270.

Mindnich, R., and Adamski, J. (2009). Zebrafish 17beta-hydroxysteroid dehydrogenases: An evolutionary perspective. Mol. Cell. Endocrinol. *301*, 20–26.

Miyoshi, Y., Ando, a, Shiba, E., Taguchi, T., Tamaki, Y., and Noguchi, S. (2001). Involvement of up-regulation of 17beta-hydroxysteroid dehydrogenase type 1 in maintenance of intratumoral high estradiol levels in postmenopausal breast cancers. Int. J. Cancer *94*, 685–689.

Moeller, G., and Adamski, J. (2006). Multifunctionality of human 17beta-hydroxysteroid dehydrogenases. Mol. Cell. Endocrinol. *248*, 47–55.

Moeller, G., and Adamski, J. (2009). Integrated view on 17beta-hydroxysteroid dehydrogenases. Mol. Cell. Endocrinol. *301*, 7–19.

Moon, Y.A., and Horton, J.D. (2003). Identification of two mammalian reductases involved in the two-carbon fatty acyl elongation cascade. J. Biol. Chem. *278*, 7335–7343.

Mustonen, M., Poutanen, M., Chotteau-Lelievre, a, de Launoit, Y., Isomaa, V., Vainio, S., Vihko, R., and Vihko, P. (1997). Ontogeny of 17beta-hydroxysteroid dehydrogenase type 2 mRNA expression in the developing mouse placenta and fetus. Mol. Cell. Endocrinol. *134*, 33–40.

Mustonen, M., Isomaa, V., Vaskivuo, T., Tapanainen, J., Poutanen, M., Stenback, F., Vihko, R., and Vihko, P. (1998a). Human 17beta-hydroxysteroid dehydrogenase type 2 messenger ribonucleic acid expression and localization in term placenta and in endometrium during the menstrual cycle. J Clin Endocrinol Metab *83*, 1319–1324.

Mustonen, M., Poutanen, M., Kellokumpu, S., De Launoit, Y., Isomaa, V., Vihko, R., and Vihko, P. (1998b). Mouse 17beta-hydroxysteroid dehydrogenase type 2 mRNA is predominantly expressed in hepatocytes and in surface epithelial cells of the gastrointestinal and urinary tracts. J. Mol. Endocrinol. *20*, 67–74.

Mäentausta, O., Svalander, P., Gemzell Danielsson, K., Bygdeman, M., and Vihko, R. (1993). The Effects of an Antiprogestin , Mifepristone, and an Antiestrogen, Tamoxifen, on Endometrial 17beta-Hydroxysteroid Dehydrogenase and Progestin and Estrogen Receptors during the Luteal Phase of the MenstrualMenstrual Cycle : An Immunohistochemical Study. J. Clin. Endocrinol. Metab. *77*, 913–918.

Narboux-Nême, N., Goïame, R., Mattêi, M.-G., Cohen-Tannoudji, M., and Wassef, M. (2012). Integration of H-2Z1, a Somatosensory Cortex-Expressed Transgene, Interferes with the Expression of the Satb1 and Tbc1d5 Flanking Genes and Affects the Differentiation of a Subset of Cortical Interneurons. J. Neurosci. *32*, 7287–7300.

Nelson, J.F., Felicio, L.S., Randall, P.K., Sims, C., and Finch, C.E. (1982). A Longitudinal Study of Estrous Cyclicity in Aging C57BL/6J Mice: I. Cycle Frequency, Length and Vaginal Cytology. Biol. Reprod. *27*, 327–339.

Nilsson, M.E., Vandenput, L., Tivesten, Å., Norlén, A.K., Lagerquist, M.K., Windahl, S.H., Börjesson, A.E., Farman, H.H., Poutanen, M., Benrick, A., et al. (2015). Measurement of a Comprehensive Sex Steroid Profile in Rodent Serum by High-Sensitive Gas Chromatography-Tandem Mass Spectrometry. Endocrinology *156*, 2492–2502.

Nokelainen, P., Puranen, T., Peltoketo, H., Orava, M., Vihko, P., and Vihko, R. (1996). Molecular cloning of mouse 17 beta-hydroxysteroid dehydrogenase type 1 and characterization of enzyme activity. Eur. J. Biochem. *236*, 482–490.

Nokelainen, P., Peltoketo, H., Vihko, R., and Vihko, P. (1998). Expression cloning of a novel estrogenic mouse 17 beta-hydroxysteroid dehydrogenase/17-ketosteroid reductase (m17HSD7), previously described as a prolactin receptor-associated protein (PRAP) in rat. Mol. Endocrinol. *12*, 1048–1059.

Nokelainen, P., Peltoketo, H., Mustonen, M., and Vihko, P. (2000). Expression of Mouse 17beta-Hydroxysteroid Dehydrogenase/17-Ketosteroid Reductase Type 7 in the Ovary , Uterus , and Placenta: Localization from Implantation to Late Pregnancy. Endocrinology *141*, 772–778.

Nolis, I.K., Mckay, D.J., Mantouvalou, E., Lomvardas, S., Merika, M., and Thanos, D. (2009). Transcription factors mediate long-range enhancer – promoter interactions. Proc Natl Acad Sci USA *106*, 20222–20227.

Normand, T., Husen, B., Leenders, F., Pelczar, H., Baert, J.-L., Begue, A., Flourens, A.-C., Adamski, J., and de Launoit, Y. (1995). Molecular Characterization of Mouse 17beta-Hydroxysteroid Dehydrogenase IV. J Steroid Biochem Mol Biol *55*, 541–548.

O'Shaughnessy, P.J., Baker, P.J., Heikkilä, M., Vainio, S., and McMahon, A.P. (2000). Localization of 17beta-Hydroxysteroid Dehydrogenase/17-Ketosteroid Reductase Isoform Expression in the Developing Mouse Testis — Androstenedione Is the Major Androgen Secreted by Fetal / Neonatal Leydig Cells. Endocrinolo *141*, 2631–2637.

Oduwole, O.O., Li, Y., Isomaa, V. V, Mantyniemi, A., Pulkka, A.E., Soini, Y., and Vihko, P.T. (2004). 17beta-Hydroxysteroid Dehydrogenase Type 1 is an Independent Prognostic Marker in Breast Cancer. Cancer Res. *64*, 7604–7609.

Ohmi, K., Kudo, L.C., Ryazantsev, S., Zhao, H.-Z., Karsten, S.L., and Neufeld, E.F. (2009). Sanfilippo syndrome type B, a lysosomal storage disease, is also a tauopathy. Proc Natl Acad Sci USA *106*, 8332–8337.

Okazaki, R., Inoue, D., Shibata, M., Saika, M., Kido, S., Ooka, H., Tomiyama, H., Sakamoto, Y., and Matsumoto, T. (2002). Estrogen promotes early osteoblast differentiation and inhibits adipocyte differentiation in mouse bone marrow stromal cell lines that express estrogen receptor (ER) alpha or beta. Endocrinology *143*, 2349–2356.

Olson, E.N., Arnold, H.H., Rigby, P.W.J., and Wold, B.J. (1996). Know your neighbors: Three phenotypes in null mutants of the myogenic bHLH gene MRF4. Cell *85*, 1–4.

Osokine, I., Hsu, R., Loeb, G.B., and McManus, M.T. (2008). Unintentional miRNA ablation is a risk factor in gene knockout studies: A short report. PLoS Genet. *4*, 4–6.

Ottaviani, E., Malagoli, D., and Franceschi, C. (2011). The evolution of the adipose tissue: A neglected enigma. Gen. Comp. Endocrinol. *174*, 1–4.

Paatela, H., Wang, F., Vihma, V., Savolainen-Peltonen, H., Mikkola, T.S., Turpeinen, U., Hämäläinen, E., Jauhiainen, M., and Tikkanen, M.J. (2016). Steroid sulfatase activity in subcutaneous and visceral adipose tissue: A comparison between pre-and postmenopausal women. Eur. J. Endocrinol. *174*, 167–175.

Palmiter, R.D., and Brinster, R.L. (1986). Germline transformation of mice. Ann Rev Genet. *20*, 465–499.

Pan, Y., Zhang, L., Liu, Q., Li, Y., Guo, H., Peng, Y., Peng, H., Tang, B., Hu, Z., Zhao, J., et al. (2016). Insertion of a knockout-first cassette in Ampd1 gene leads to neonatal death by disruption of neighboring genes expression. Sci. Rep. *6*, 35970.

Parmer, T.G., Duan, W.R., Albarracin, C.T., Khan, I., and Gibori, G. (1992). Hormonal and Immunological Characterization of the 32 Kilodalton Ovarian-Spesific Protein. Endocrinology *131*, 2213–2221.

Pelletier, G., Luu-The, V., Li, S., Ren, L., and Labrie, F. (2004). Localization of 17beta-hydroxysteroid dehydrogenase type 1 mRNA in mouse tissues. J. Mol. Endocrinol. *33*, 459–465.

Pelletier, G., Luu-The, V., Li, S., and Labrie, F. (2005). Localization of type 8 17beta-hydroxysteroid dehydrogenase mRNA in mouse tissues as studied by in situ hybridization. Cell Tissue Res. *320*, 393–398.

Peltoketo, H., Isomaa, V., Mäentausta, O., and Vihko, R. (1988). Complete amino acid

sequence of human placental 17 beta-hydroxysteroid dehydrogenase deduced from cDNA. FEBS Lett. *239*, 73–77.

Peltoketo, H., Isomaa, V., and Vihko, R. (1992a). Genomic organization and DNA sequences of human 17 beta-hydroxysteroid dehydrogenase genes and flanking regions. Localization of multiple Alu sequences and putative cis-acting elements. Eur. J. Biochem. *209*, 459–466.

Peltoketo, H., Isomaa, V., and Vihko, R. (1992b). Genomic organization and DNA sequences of human 17beta-hydroxysteroid dehydrogenase genes and flanking regions. Eur. J. Biochem. *209*, 459–466.

Peltoketo, H., Luu-The, V., Simard, J., and Adamski, J. (1999). 17beta-Hydroxysteroid dehydrogenase (HSD)/17-ketosteroid reductase (KSR) family; nomenclature and main characteristics of the 17HSD/KSR enzymes. J. Mol. Endocrinol. *23*, 1–11.

Pham, C.T., MacIvor, D.M., Hug, B.A., Heusel, J.W., and Ley, T.J. (1996). Long-range disruption of gene expression by a selectable marker cassette. Proc Natl Acad Sci USA *93*, 13090–13095.

Piao, Y.S., Peltoketo, H., Oikarinen, J., and Vihko, R. (1995). Coordination of transcription of the human 17 beta- hydroxysteroid dehydrogenase type 1 gene (EDH17B2) by a cell- specific enhancer and a silencer: identification of a retinoic acid response element. Mol. Endocrinol. *9*, 1633–1644.

Poirier, D. (2011). Contribution to the development of inhibitors of 17beta-hydroxysteroid dehydrogenase types 1 and 7: Key tools for studying and treating estrogen-dependent diseases. J. Steroid Biochem. Mol. Biol. *125*, 83–94.

Polyzos, S. a, Kountouras, J., Tsatsoulis, A., Zafeiriadou, E., Katsiki, E., Patsiaoura, K., Zavos, C., Anastasiadou, V. V, and Slavakis, A. (2013). Sex steroids and sex hormone-binding globulin in postmenopausal women with nonalcoholic fatty liver disease. Hormones (Athens). *12*, 405–416.

Poutanen, M., Isomaa, V., Kainulainen, K., and Vihko, R. (1990). Progestin induction of 17-beta-hydroxysteroid dehydrogenase enzyme protein in the T47D human breast cancer cell line. Int. J. Cancer *46*, 897–901.

Poutanen, M., Miettinen, M., and Vihko, R. (1993). Differential estrogen substrate specificities for transiently expressed human placental 17beta-Hydroxysteroid dehydrogenase and an endogenous enzyme expressed in cultured COS-m6 cells. Endocrinology *133*, 2639–2644.

Pulit, S.L., Karaderi, T., and Lindgren, C.M. (2017). Sexual dimorphisms in genetic loci linked to body fat distribution. Biosci. Rep. *37*, 1–10.

Puranen, T., Poutanen, M., Ghosh, D., Vihko, R., and Vihko, P. (1997). Origin of Substrate Specificity of Human and Rat 17β-Hydroxysteroid Dehydrogenase Type 1, Using Chimeric Enzymes and Site-Directed Substitutions. Endocrinology *138*, 3532–3539.

Ramirez-Solis, R., Zheng, H., Whiting, J., Krumlauf, R., and Bradley, A. (1993). Hoxb-4 (Hox-2.6) mutant mice show homeotic transformation of a cervical vertebra and defects in the closure of the sternal rudiments. Cell *73*, 279–294.

Rantakari, P., Strauss, L., Kiviranta, R., Lagerbohm, H., Paviala, J., Holopainen, I., Vainio, S., Pakarinen, P., and Poutanen, M. (2008). Placenta defects and embryonic lethality resulting from disruption of mouse hydroxysteroid (17-beta) dehydrogenase 2 gene. Mol. Endocrinol. *22*, 665–675.

Rantakari, P., Lagerbohm, H., Kaimainen, M., Suomela, J.P., Strauss, L., Sainio, K., Pakarinen, P., and Poutanen, M. (2010). Hydroxysteroid (17beta) dehydrogenase 12 is essential for mouse organogenesis and embryonic survival. Endocrinology *151*, 1893–1901.

Rautou, P.-E., Mansouri, A., Lebrec, Didier, Durand, F., Valla, D., and Moreau, R. (2011). Autophagy in liver diseases. J. UOEH *33*, 337–344.

Reddy, S., DeGregori, J. V, von Melchner, H., and Ruley, H.E. (1991). Retrovirus promoter-trap vector to induce lacZ gene fusions in mammalian cells. J. Virol. *65*, 1507–1515.

Rodgers, R.J., Irving-Rodgers, H.F., Van Wezel, I.L., Krupa, M., and Lavranos, T.C. (2001). Dynamics of the membrana granulosa during expansion of the ovarian follicular antrum. Mol. Cell. Endocrinol. *171*, 41–48.

Rodriguez, A. (2004). Identification of Mammalian microRNA Host Genes and Transcription Units. Genome Res. *14*, 1902–1910.

Rodriguez, I., Araki, K., Khatib, K., Martinou, J.C., and Vassalli, P. (1997). Mouse vaginal opening is an apoptosis-dependent process which can be prevented by the overexpression of Bcl2. Dev. Biol. *184*, 115–121.

Rogers, A.B., and Dintzis, R.Z. (2012). Liver and Gallbladder. In Comparative Anatomy and Histology, (Elsevier), pp. 193–201.

Sakurai, N., Miki, Y., Suzuki, T., Watanabe, K., Narita, T., Ando, K., Yung, T.M.C., Aoki, D., Sasano, H., and Handa, H. (2006). Systemic

distribution and tissue localizations of human 17beta-hydroxysteroid dehydrogenase type 12. J. Steroid Biochem. Mol. Biol. *99*, 174–181.

Saloniemi, T., Lamminen, T., Huhtinen, K., Welsh, M., Saunders, P., Kujari, H., and Poutanen, M. (2007). Activation of androgens by hydroxysteroid (17beta) dehydrogenase 1 in vivo as a cause of prenatal masculinization and ovarian benign serous cystadenomas. Mol. Endocrinol. *21*, 2627–2636.

Sander, J.D., and Joung, J.K. (2014). CRISPR-Cas systems for editing, regulating and targeting genomes. Nat. Biotechnol. *32*, 347–355.

Sano, T., Hirasawa, G., Takeyama, J., and Darnel, A.D. (2001). Expression and Enzyme Activity in the Human Gastrointestinal Tract. *491*, 485–491.

Sardiello, M., Palmieri, M., di Ronza, A., Medina, D.L., Valenza, M., Gennarino, V., Di Malta, C., Donaudy, F., EMbrione, V., Polishchuk, R., et al. (2009). A Gene Network Regulating Lysosomal Biogenesis and Function. Science *325*, 473–477.

Sarjeant, K., and Stephens, J.M. (2012). Adipogenesis. Cold Spring Harb Perspect Biol *4*, 1–20.

Sasaki, T., Kazuko, S., Masue, M., Fukuda, S., Tomatsu, S., and Orii, T. (1991). Purification and partial characterization of alpha-N-acetylglucosaminidase from human liver. J Biochem *110*, 842–846.

Scacheri, P.C., Crabtree, J.S., Novotny, E.A., Garrett-Beal, L., Chen, A., Edgemon, K.A., Marx, S.J., Spiegel, A.M., Chandrasekharappa, S.C., and Collins, F.S. (2001). Bidirectional transcriptional activity of PGK-neomycin and unexpected embryonic lethality in heterozygote chimeric knockout mice. Genesis *30*, 259–263.

Serke, H., Vilser, C., Nowicki, M., Hmeidan, F.A., Blumenauer, V., Hummitzsch, K., Lösche, A., and Spanel-Borowski, K. (2009). Granulosa cell subtypes respond by autophagy or cell death to oxLDL-dependent activation of the oxidized lipoprotein receptor 1 and toll-like 4 receptor. Autophagy *5*, 991–1003.

Shehu, A., Mao, J., Gibori, G.B., Halperin, J., Le, J., Devi, Y.S., Merrill, B., Kiyokawa, H., and Gibori, G. (2008). Prolactin receptor-associated protein/17beta-hydroxysteroid dehydrogenase type 7 gene (Hsd17b7) plays a crucial role in embryonic development and fetal survival. Mol. Endocrinol. *22*, 2268–2277.

Shen, M., and Shi, H. (2015). Sex Hormones and Their Receptors Regulate Liver Energy Homeostasis. Int. J. Endocrinol. *2015*, 1–12.

Shibuya, R., Suzuki, T., Miki, Y., Yoshida, K., Moriya, T., Ono, K., Akahira, J.I., Ishida, T., Hirakawa, H., Evans, D.B., et al. (2008). Intratumoral concentration of sex steroids and expression of sex steroid-producing enzymes in ductal carcinoma in situ of human breast. Endocr. Relat. Cancer *15*, 113–124.

Singh, R., and Cuervo, A.M. (2012). Lipophagy: Connecting autophagy and lipid metabolism. Int. J. Cell Biol. *2012*.

Singh, R., Xiang, Y., Wang, Y., Baikati, K., Cuervo, A.M., Luu, Y.K., Tang, Y., Pessin, J.E., Schwartz, G.J., and Czaja, M.J. (2009). Autophagy regulates adipose mass and differentiation in mice. J. Clin. Invest. *119*, 3329–3339.

Smith, M.S., Freeman, M.E., and Neill, J.D. (1975). The control of progesterone secretion during the estrus cycle and early pseudopregnancy in the rat: gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. Endocrinology *96*, 219–226.

Smithies, O., Gregg, R.G., Boggs, S.S., Koralewski, M.A., and Kucherlapati, R.S. (1985). Insertion of DNA sequences into the human chromosomal beta-globin locus by homologous recombination. Nature *317*, 230–234.

Šmuc, T., Pucelj, M.R., Šinkovec, J., Husen, B., Thole, H., and Rižner, T.L. (2007). Expression analysis of the genes involved in estradiol and progesterone action in human ovarian endometriosis. Gynecol. Endocrinol. *23*, 105–111.

Sproul, D., Gilbert, N., and Bickmore, W. a (2005). The role of chromatin structure in regulating the expression of clustered genes. Nat. Rev. Genet. *6*, 775–781.

Stanbrough, M., Bubley, G.J., Ross, K., Golub, T.R., Rubin, M.A., Penning, T.M., Febbo, P.G., and Balk, S.P. (2006). Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res. *66*, 2815–2825.

Stanford, W.L., Cohn, J.B., and Cordes, S.P. (2001). Gene-trap mutagenesis: past, present and beyond. Nat. Rev. Genet. *2*, 756–768.

Stocco, C., Telleria, C., and Gibori, G. (2007). The molecular control of corpus luteum formation, function, and regression. Endocr. Rev. *28*, 117–149.

Strauss, J.F., and Williams, C.J. (2014). The Ovarian Life Cycle. In Yen and Jaffe's Reproductive Endocrinology: Seventh Edition, (Elsevier), pp. 157–191.

Strauss, J.F., Modi, B., and McAllister, J.M. (2014). Defects in Ovarian Steroid Hormone Biosynthesis. In Cellular Endocrinology in Health and Disease, (Elsevier), pp. 285–308.

Su, J., Lin, M.I.N., and Napoli, J.L. (1999). Complementary Deoxyribonucleic Acid Cloning and Enzymatic Characterization of a Novel 17beta/3alpha-Hydroxysteroid/retinoid short chain dehydrogenase/reductase. Endocrinology *140*, 5275–5284.

Su, W., Wang, Y., Jia, X., Wu, W., Li, L., Tian, X., Li, S., Wang, C., Xu, H., Cao, J., et al. (2014). Comparative proteomic study reveals 17β-HSD13 as a pathogenic protein in nonalcoholic fatty liver disease. Proc Natl Acad Sci USA *111*, 11437–11442.

Suriawinata, A., and Thung, S. (2011). Chronic Liver disorders. In Liver Pathology. An Atlas and Concise Guide., (Springer), pp. 74–123.

Taniguchi, F., Couse, J.F., Rodriguez, K.F., Emmen, J.M.A., Poirier, D., and Korach, K.S. (2007). Estrogen receptor-α mediates an intraovarian negative feedback loop on thecal cell steroidogenesis via modulation of CYP17A1 (cytochrome P450, steroid 17α-hydroxylase/17,20 lyase) expression. FASEB J. *21*, 586–595.

Tchernof, A., Mansour, M.F., Pelletier, M., Boulet, M.M., Nadeau, M., and Luu-The, V. (2015). Updated survey of the steroid-converting enzymes in human adipose tissues. J Steroid Biochem Mol Biol *147*, 56–69.

Tremblay, Y., Ringler, G.E., Morel, Y., Mohandas, T.K., Labrie, F., Straus, J.F., and Miller, W.L. (1989). Regulation of the gene for estrogenic 17-ketosteroid reductase lying on chromosome 17cen→q25. J. Biol. Chem. *264*, 20458–20462.

Tschaharganeh, D.F., Lowe, S.W., Garippa, R.J., and Livshits, G. (2016). Using CRISPR/Cas to study gene function and model disease in vivo. FEBS J. *283*, 3194–3203.

Törn, S., Nokelainen, P., Kurkela, R., Pulkka, A., Menjivar, M., Ghosh, S., Coca-Prados, M., Peltoketo, H., Isomaa, V., and Vihko, P. (2003). Production, purification, and functional analysis of recombinant human and mouse 17β-hydroxysteroid dehydrogenase type 7. Biochem. Biophys. Res. Commun. *305*, 37–45.

Ueno, T., and Komatsu, M. (2017). Autophagy in the liver: functions in health and disease. Nat. Rev. Gastroenterol. Hepatol. *14*, 170–184.

Valenzuela, D.M., Murphy, A.J., Frendewey, D., Gale, N.W., Economides, A.N., Auerbach, W., Poueymirou, W.T., Adams, N.C., Rojas, J., Yasenchak, J., et al. (2003). High-throughput engineering of the mouse genome coupled with high-resolution expression analysis. Nat. Biotechnol. *21*, 652–659.

Vaskivuo, T.E., Mäentausta, M., Törn, S., Oduwole, O., Lönnberg, A., Herva, R., Isomaa, V., and Tapanainen, J.S. (2005). Estrogen receptors and estrogen-metabolizing enzymes in human ovaries during fetal development. J. Clin. Endocrinol. Metab. *90*, 3752–3756.

Venkatesan, R., Sah-Teli, S.K., Awoniyi, L.O., Jiang, G., Prus, P., Kastaniotis, A.J., Hiltunen, J.K., Wierenga, R.K., and Chen, Z. (2014). Insights into mitochondrial fatty acid synthesis from the structure of heterotetrameric 3-ketoacyl-ACP reductase/3R-hydroxyacyl-CoA dehydrogenase. Nat. Commun. *5*, 1–12.

Ventura, A., Kirsch, D.G., McLaughlin, M.E., Tuveson, D.A., Grimm, J., Lintault, L., Newman, J., Reczek, E.E., Weissleder, R., and Jacks, T. (2007). Restoration of p53 function leads to tumour regression in vivo. Nature *445*, 661–665.

Vigouroux, C., Caron-Debarle, M., Le Dour, C., Magré, J., and Capeau, J. (2011). Molecular mechanisms of human lipodystrophies: From adipocyte lipid droplet to oxidative stress and lipotoxicity. Int. J. Biochem. Cell Biol. *43*, 862–876.

Vihko, P., Herrala, A., Härkönen, P., Isomaa, V., Kaija, H., Kurkela, R., Li, Y., Patrikainen, L., Pulkka, A., Soronen, P., et al. (2005). Enzymes as modulators in malignant transformation. J. Steroid Biochem. Mol. Biol. *93*, 277–283.

Vihko, P., Herrala, A., Härkönen, P., Isomaa, V., Kaija, H., Kurkela, R., and Pulkka, A. (2006). Control of cell proliferation by steroids: The role of 17HSDs. Mol. Cell. Endocrinol. *248*, 141–148.

Walmer, D., Wrona, M., Hughes, C., and Nelson, K.G. (1992). Lactoferrin Tract during the Natural Estrous Cycle : Correlation with Circulating Estradiol and Progesterone. Endocrinology *131*, 1458–1466.

Wang, X., Gérard, C., Thériault, J.F., Poirier, D., Doillon, C.J., and Lin, S.X. (2015). Synergistic control of sex hormones by 17??-HSD type 7: A novel target for estrogen-dependent breast cancer. J. Mol. Cell Biol. *7*, 568–579.

Weber, B., Blanch, L., Clements, P.R., Scott, H.S., and Hopwood, J.J. (1996). Cloning and expression of the gene involved in Sanfilippo B syndrome (mucopolysaccharidosis III B). Hum. Mol. Genet. *5*, 771–777.

Weckman, A., Di Ieva, A., Rotondo, F., Syro, L. V., Ortiz, L.D., Kovacs, K., and Cusimano, M.D. (2013). Autophagy in the endocrine glands. J. Mol. Endocrinol. *52*.

West, D.B., Engelhard, E.K., Adkisson, M., Nava, A.J., Kirov, J. V., Cipollone, A., Willis, B., Rapp, J., de Jong, P.J., and Lloyd, K.C. (2016). Transcriptome Analysis of Targeted Mouse Mutations Reveals the Topography of Local

Changes in Gene Expression. PLoS Genet. *12*, 1–19.

Whitelock, J.M., and Iozzo, R. V. (2005). Heparan sulfate: A complex polymer charged with biological activity. Chem. Rev. *105*, 2745–2764.

Winqvist, R., Peltoketo, H., Isomaa, V., Grzeschik, K.-H., Mannermaa, A., and Vihko, R. (1990). The gene for 17beta-hydroxysteroid dehydrogenase maps to human chromosome 17, bands q12-q21, and shows an RFLP with. Hum. Genet. *3104*, 473–476.

Woloszynek, J.C., Coleman, T., Semenkovich, C.F., and Sands, M.S. (2007). Lysosomal dysfunction results in altered energy balance. J Biol Chem *282*, 35765–35771.

Wu, L., Einstein, M., Geissler, W.M., and Chan, H.K. (1993). Expression Cloning and Characterization of Human 178- Hydroxysteroid Dehydrogenase Type 2 , a Microsomal Enzyme Possessing 20a-Hydroxysteroid Dehydrogenase Activity. J. Biol. Chem. *268*, 12964–12969.

Wu, X., Lukacik, P., Kavanagh, K.L., and Oppermann, U. (2007). SDR-type human hydroxysteroid dehydrogenases involved in steroid hormone activation. Mol. Cell. Endocrinol. *265–266*, 71–76.

Yang, S.-Y., He, X.-Y., Olpin, S.E., Sutton, V.R., McMenamin, J., Philipp, M., Denman, R.B., and Malik, M. (2009). Mental retardation linked to mutations in the HSD17B10 gene interfering with neurosteroid and isoleucine metabolism. Proc Natl Acad Sci USA *106*, 14820–14824.

Yang, S.Y., He, X.Y., and Miller, D. (2007). HSD17B10: A gene involved in cognitive function through metabolism of isoleucine and neuroactive steroids. Mol. Genet. Metab. *92*, 36–42.

Yang, S.Y., He, X.Y., Isaacs, C., Dobkin, C., Miller, D., and Philipp, M. (2014). Roles of 17beta-hydroxysteroid dehydrogenase type 10 in neurodegenerative disorders. J. Steroid Biochem. Mol. Biol. *143*, 460–472.

Yang, X., Yang, X., Schadt, E.E., Schadt, E.E., Wang, S., Wang, S., Wang, H., Wang, H., Arnold, A.P., Arnold, A.P., et al. (2006). Tissue-specific expression and regulation of sexually dimorphic genes in mice\r10.1101/gr.5217506. Genome Res. *16*, 995–1004.

Yates, A., Akanni, W., Amode, M.R., Barrell, D., Billis, K., Carvalho-Silva, D., Cummins, C., Clapham, P., Fitzgerald, S., Gil, L., et al. (2016). Ensembl 2016. Nucleic Acids Res. *44*, D710–D716.

Zabidi, M.A., and Stark, A. (2016). Regulatory Enhancer–Core-Promoter Communication via Transcription Factors and Cofactors. Trends Genet. *32*, 801–814.

Zhao, H.G., Li, H.H., Bach, G., Schmidtchen, A., and Neufeld, E.F. (1996). The molecular basis of Sanfilippo syndrome type B. Proc Natl Acad Sci USA *93*, 6101–6105.

Zhong, L., Parmer, T.G., Robertson, M.C., and Gibori, G. (1997). Prolactin-Mediated Inhibition of 20α-Hydroxysteroid Dehydrogenase Gene Expression and the Tyrosine Kinase System. Biochem. Biophys. Res. Commun. *235*, 587–592.

Zhongyi, S., Rantakari, P., Lamminen, T., Toppari, J., and Poutanen, M. (2007). Transgenic male mice expressing human hydroxysteroid dehydrogenase 2 indicate a role for the enzyme independent of its action on sex steroids. Endocrinology *148*, 3827–3836.

Zhu, S.J., Li, Y., Li, H., Wang, Y.L., Xiao, Z.J., Vihko, P., and Piao, Y.S. (2002). Retinoic acids promote the action of aromatase and 17β-hydroxysteroid dehydrogenase type 1 on the biosynthesis of 17β-estradiol in placental cells. J. Endocrinol. *172*, 31–43.

Zuniga, A., Michos, O., Spitz, F., Haramis, An.-P., Panman, L., Galli, A., Vintersen, K., Klasen, C., Mansfield, W., Kuc, S., et al. (2004). Mouse limb deformity mutations disrupt a global control region within the large regulatory landscape required for Gremlin expression. Genes Dev. *18*, 1553–1564.

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